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Chemical Analyses for Water Pollutants

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CHEMICAL ANALYSES FOR WATER POLLUTANTS

TRAINING MANUAL

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER PROGRAM OPERATIONS

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This training manual contains outlined information used by lecturers when presenting topics in EPA-NTOTC Course 100.3, "Chemical Analyses for Water Pollutants." The contents concern individual constituents found in water (e.g. dissolved oxygen) and information useful to analysts (e.g. statistics). A section of laboratory procedures adapted for class groups is included.

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CHEMICAL ANALYSES FOR WATER POLLUTANTS

This course is designed for chemists or technicians who will perform chemical analyses for water pollutants.

The student will learn about analyses listed in the guidelines developed pursuant to Section 304(g) of the Federal Water Pollution Control Act Amendments of 1972 for NPDES permit applications and reports and also for certifications issued by the States.

The student will learn theoretical concepts and perform laboratory exercises pertaining to the following: Acidity and Alkalinity Determinations, pH Measurement, Biochemical Oxygen Demand, Dissolved Oxygen (Winkler and Probe), Calcium and Magnesium Hardness Determination, Nitrogen Analyses (Ammonia, Kjeldahl, Nitrate, Nitrite), Phosphorus Determinations, Total Organic Carbon Analysis, Total Solids Determination.

Discussion periods are scheduled after each laboratory assignment is completed. Time is divided about equally between classroom and laboratory work.

Persons attending should have fundamental knowledge of inorganic chemistry and quantitative analysis. They should also be able to use an analytical balance, volumetric glassware and titration assemblies.

U. S. ENVIRONMENTAL PROTECTION AGENCY
Office of Water Program Operations
TRAINING PROGRAM

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FOREWORD

These manuals are prepared for reference use of students enrolled in scheduled training courses of the Office of Water Program Operations, Environmental Protection Agency.

> Due to the limited availability of the manuals it is not appropriate to cite them as technical references in bibliographies or other forms of publication.
>
> References to products and manufacturers are for illustration only; such references do not imply product endorsement by the Office of Water Program Operations, Environmental Protection Agency.

The reference outlines in this manual have been selected and developed with a goal of providing the student with a fund of the best available current information pertinent to the subject matter of the course. Individual instructors may provide additional material to cover special aspects of their own presentations.

This manual will be useful to anyone who has need for information on the subjects covered. However, it should be understood that the manual will have its greatest value as an adjunct to classroom presentations. The inherent advantages of classroom presentation is in the give-and-take discussions and exchange of information between and among students and the instructional staff.

Constructive suggestions for improvement in the coverage, content, and format of the manual are solicited and will be given full consideration.

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SAMPLE HANDLING - FIELD THROUGH LABORATORY

I PLANNING A SAMPLING PROGRAM

A Factors to Consider:

1 Locating sampling sites

2 Sampling equipment

3 Type of sample required

a grab
b composite

4 Amount of sample required

5 Frequency of collection

6 Preservation measures, if any

B Decisive Criteria

1 Nature of the sample source

2 Stability of constituent(s) to be measured

3 Ultimate use of data

II REPRESENTATIVE SAMPLES

If a sample is to provide meaningful and valid data about the parent population, it must be representative of the conditions existing in that parent source at the sampling location.

A The container should be rinsed two or three times with the water to be collected.

B Compositing Samples

1 For some sources, a composite of samples is made which will represent the average situation for stable constituents.

2 The nature of the constituent to be determined may require a series of separate samples.

C The equipment used to collect the sample is an important factor to consider. ASTM(1) has a detailed section on various sampling devices and techniques.

D Great care must be exercised when collecting samples in sludge or mud areas and near benthic deposits. No definite procedure can be given, but careful effort should be made to obtain a representative sample.

III SAMPLE IDENTIFICATION

A Each sample must be unmistakably identified, preferably with a tag or label. The required information should be planned in advance.

B An information form preprinted on the tags or labels provides uniformity of sample records, assists the sampler, and helps ensure that vital information will not be omitted.

C Useful Identification Information includes:

1 sample identity code
2 signature of sampler
3 signature of witness
4 description of sampling location detailed enough to accommodate reproducible sampling. (It may be more convenient to record the details in the field record book).
5 sampling equipment used
6 date of collection
7 time of collection
8 type of sample (grab or composite)
9 water temperature
10 sampling conditions such as weather, water level, flow rate of source, etc.
11 any preservative additions or techniques
12 record of any determinations done in the field
13 type of analyses to be done in laboratory

IV SAMPLE CONTAINERS

A Available Materials

1 glass
2 plastic
3 hard rubber

B Considerations

1 Nature of the sample - Organics attack polyethylene.

2 Nature of constituent(s) to be determined - Cations can adsorb readily on some plastics and on certain glassware. Metal or aluminum foil cap liners can interfere with metal analyses.

3 Preservatives to be used - Mineral acids attack some plastics.

4 Mailing Requirements - Containers should be large enough to allow extra volume for effects of temperature changes during transit. All caps should be securely in place. Glass containers must be protected against breakage. Styrofoam linings are useful for protecting glassware.

C Preliminary Check

Any question of possible interferences related to the sample container should be resolved before the study begins. A preliminary check should be made using corresponding sample materials, containers, preservatives and analysis.

D Cleaning

If new containers are to be used, preliminary cleaning is usually not necessary.

If the sample containers have been used previously, they should be carefully cleaned before use.

There are several cleaning methods available. Choosing the best method involves careful consideration of the nature of the sample and of the constituent(s) to be determined.

1 Phosphate detergents should not be used to clean containers for phosphorus samples.

2 Traces of dichromate cleaning solution will interfere with metal analyses.

E Storage

Sample containers should be stored and transported in a manner to assure their readiness for use.

V SAMPLE PRESERVATION

Every effort should be made to achieve the shortest possible interval between sample collection and analyses. If there must be a delay and it is long enough to produce significant changes in the sample, preservation measures are required.

At best, however, preservation efforts can only retard changes that inevitably continue after the sample is removed from the parent population.

A Functions

Methods of preservation are relatively limited. The primary functions of those employed are:

1 to retard biological action
2 to retard precipitation or the hydrolysis of chemical compounds and complexes
3 to reduce volatility of constituents

B General Methods

1 pH control - This affects precipitation of metals, salt formation and can inhibit bacterial action.

2 Chemical Addition - The choice of chemical depends on the change to be controlled.

Mercuric chloride is commonly used as a bacterial inhibitor. Disposal of the mercury-containing samples is a problem and efforts to find a substitute for this toxicant are underway.

To dispose of solutions of inorganic mercury salts, a recommended procedure is to capture and retain the mercury salts as the sulfide at a high pH. Several firms have tentatively agreed to accept the mercury sulfide for re-processing after preliminary conditions are met.(4)

3 Refrigeration and Freezing - This is the best preservation technique available, but it is not applicable to all types of samples. It is not always a practical technique for field operations.

C Specific Methods

The EPA Methods Manual(2) includes a table summarizing the holding times and preservation techniques for several analytical procedures. This information also can be found in the standard references(1, 2, 3) as part of the presentation of the individual procedures.

VI METHODS OF ANALYSIS

Standard reference books of analytical procedures to determine the physical and chemical characteristics of various types of water samples are available.

A EPA Methods Manual

The Methods Development and Quality Assurance Research Laboratory of the Environmental Protection Agency, has published a manual of analytical procedures to provide methodology for monitoring the quality of our Nation's Waters and to determine the impact of waste discharges. The title of this manual is "Methods for Chemcal Analysis of Water and Wastes."(2)

For some procedures, the analyst is referred to Standard Methods and/or to ASTM Standards.

B Standard Methods

The American Public Health Association, the American Water Works Association and the Water Pollution Control Federation prepare and publish a volume describing methods of water analysis. These include physical and chemical procedures. The title of this book is "Standard Methods for the Examination of Water and Wastewater."(3)

C ASTM Standards

The American Society for Testing and Materials publishes an annual "book" of specifications and methods for testing materials. The "book" currently consists of 33 parts. The part applicable to water is a book titled, "Annual Book of ASTM Standards, Part 23, Water; Atmospheric Analysis".(1)

D Other References

Current literature and other books of analytical procedures with related information are available to the analyst.

E NPDES Methodology

When gathering data for National Pollutant Discharge Elimination System report purposes, the analyst must consult the Federal Register for a listing of approved analytical methodology. There he will be directed to pages in the above cited reference books where acceptable procedures can be found. The Federal Register also provides information concerning the protocol for obtaining approval to use analytical procedures other than those listed.

VII ORDER OF ANALYSES

The ideal situation is to perform all analyses shortly after sample collection. In the practical order, this is rarely possible. The allowable holding time for preserved samples is the basis for scheduling analyses.

A The allowable holding time for samples depends on the nature of the sample, the stability of the constituent(s) to be determined and the conditions of storage.

1 For some constituents and physical values, immediate determination is required, e.g. dissolved oxygen, pH.

2 Using preservation techniques, the holding times for other determinations range from 6 hours (BOD) to 7 days (COD). Metals may be held up to 6 months.(2)

3 The EPA Methods Manual(2) includes a table summarizing holding times and preservation techniques for several analytical procedures. This information can also be found in the standard references(1, 2, 3) as part of the presentation of the individual procedures.

4 If dissolved concentrations are sought, filtration should be done in the field if at all possible. Otherwise, the sample is filtered as soon as it is received in the laboratory. A 0.45 micron membrane filter is recommended for reproducible filtration.

B The time interval between collection and analysis is important and should be recorded in the laboratory record book.

VIII RECORD KEEPING

The importance of maintaining a bound, legible record of pertinent information on samples cannot be over-emphasized.

A Field Operations

A bound notebook should be used. Information that should be recorded includes:

1 Sample identification records (See Part III)

2 Any information requested by the analyst as significant

3 Details of sample preservation

4 A complete record of data on any determinations done in the field. (See B, next)

5 Shipping details and records

B Laboratory Operations

Samples should be logged in as soon as received and the analyses performed as soon as possible.

A bound notebook should be used. Preprinted data forms provide uniformity of records and help ensure that required information will be recorded. Such sheets should be permanently bound.

Items in the laboratory notebook would include:

1 sample identifying code
2 date and time of collection
3 date and time of analysis
4 the analytical method used
5 any deviations from the analytical method used and why this was done
6 data obtained during analysis
7 results of quality control checks on the analysis
8 any information useful to those who interpret and use the data
9 signature of the analyst

IX SUMMARY

Valid data can be obtained only from a representative sample, unmistakably identified, carefully collected and stored. A skilled analyst, using approved methods of analyses and performing the determinations within the prescribed time limits, can produce data for the sample. This data will be of value only if a written record exists to verify sample history from the field through the laboratory.

REFERENCES

1 ASTM Standards, Part 23, Water; Atmospheric Analysis.

2 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL, Cincinnati, OH 45268, 1974.

3 Standard Methods for the Examination of Water and Wastewater, 13th edition, APHA-AWWA-WPCF, 1971.

4 Dean, R., Williams, R. and Wise, R., Disposal of Mercury Wastes from Water Laboratories, Environmental Science and Technology, October, 1971.

This outline was prepared by A. Donahue, Chemist, National Training Center, MPOD, OWPO, EPA, Cincinnati, Ohio 45268.

Descriptors: On-Site Data Collections, On-Site Investigations, Planning, Handling, Sample, Sampling, Water Sampling, Surface Waters, Preservation, Wastewater

STATISTICS FOR CHEMISTS

I INTRODUCTION

A Statistics may be defined, for our purpose, as a collection of methods which have been developed for handling numerical data pertaining to samples or portions of entire populations.

B The statistical methods with which we will concern ourselves deal with the presentation and analysis of numerical data from samples.

II FREQUENCY

A Definitions

1 Frequency - indicates how many times a particular score occurs in a collection of data

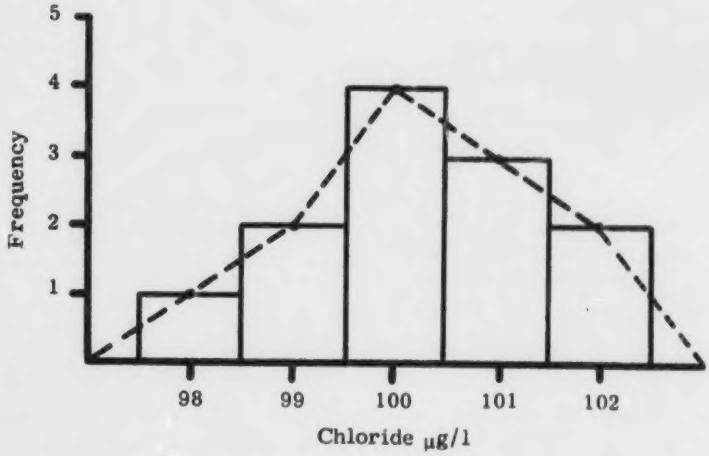
2 Frequency table - a tabular arrangement of data, ranked in ascending or descending order of magnitude, together with the corresponding frequencies

3 Frequency histogram - a set of rectangles having bases on a horizontal axis with centers at the given scores and heights equal to the corresponding frequencies (See Figure 1)

4 Frequency polygon - a line graph of frequencies plotted against scores (can be obtained by connecting midpoints of tops of rectangles in the frequency histogram) (See Figure 1)

Figure 1

Frequency Histogram & Frequency Polygon



B Application

Consider the application of the above definitions to the following set of data, obtained from twelve determinations for chloride in water.

Results (µg/l)		
100	101	99
101	100	100
99	102	100
98	101	102

Table 1

Frequency Table

Chloride (µg/l)	Frequency
98	1
99	2
100	4
101	3
102	2

III MEASURES OF CENTRAL TENDENCY

A Definitions

1 Central tendency - the tendency of values to cluster about a particular value in the distribution

2 Mode - that value which occurs most frequently

3 Median - midpoint of an array of scores. If there is an odd number of observations, n, the median is $X_{\frac{n+1}{2}}$ where $X_{\frac{n+1}{2}}$ represents the $\frac{n+1}{2}$ value in the frequency distribution. If there is an even number of observations the median is $\frac{X_{\frac{n}{2}} + X_{\frac{n}{2}+1}}{2}$, the average of the middle two scores.

4 Mean - arithmetic average of all the values in the sample distribution, denoted by $\overline{X}$. The formula for calculating the sample mean is

$$\overline{X} = \frac{X_1 + X_2 + X_3 \ldots X_n}{n}$$

$$\overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

$\overline{X} = \frac{\Sigma X_i}{n}$ where there are n number of values.

B Aids in calculation of the mean

Application of the following two statements can reduce errors and amount of time spent in calculating the mean of a distribution.

1 Adding or subtracting a constant to or from each score in a distribution is equivalent to adding or subtracting the same constant to or from the mean of the distribution. Thus the following formula:

$\overline{X}_c = \overline{X} \pm C$ where the X_i's are the values in the distribution with mean $\overline{X}$, and the $X_i \pm C$'s are the values in the distribution with mean $\overline{X}_c$.

2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the mean of the distribution by the same constant. Thus the following formulas:

(1) $\overline{X}_c = C\overline{X}$

or

(2) $\overline{X}_c = \frac{\overline{X}}{C}$ where the X_i's are the values in the distribution with mean $\overline{X}$,

and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with mean $\bar{X}_c$

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in Table 1.

1 Mode = 100

2 Median $= \frac{X_{\frac{n}{2}} + X_{\frac{n}{2}+1}}{2} = \frac{X_6 + X_7}{2}$

$= \frac{100 + 100}{2} = 100$

3 Mean $= \frac{\Sigma X_i}{n}$

$= \frac{98 + 2\,(99) + 4\,(100) + 3\,(101) + 2\,(102)}{12}$

$= 100.25$

4 Aid in Calculation

Consulting Table 1 and observing that the values are in the neighborhood of 100 we might subtract 100 from each score and obtain the following distribution:

Table 2

Frequency Table

Chloride (μg/l)	Frequency
-2	1
-1	2
0	4
1	3
2	2

Denote the mean of the distribution in Table 1 by $\bar{X}_c$. If we add 100 to each score in the distribution in Table 2, we obtain the scores in the distribution in Table 1; likewise if we add 100 to the mean, X, of the distribution in Table 2, we obtain the mean, $\bar{X}_c$, of the distribution in Table 1.

Thus $\bar{X}_c = \bar{X} + 100$

$$\bar{X}_c = \frac{\Sigma X}{n} + 100$$

$$\bar{X}_c = \frac{1(-2) + 2(-1) + 4(0) + 3(1) + 2(2)}{12} + 100$$

$$\bar{X}_c = .25 + 100 = 100.25$$

IV MEASURES OF DISPERSION

A Definitions

1 Dispersion - spread or variability of observations in a distribution

2 Range - the difference between the highest value and the lowest value

$R = \max - \min$

3 Average deviation - the sum of the deviations of the values from their mean, without regard to sign, divided by the total number of data values (n)

The formula for calculating the average deviation is:

$$d = \frac{\Sigma|X_i - \bar{X}|}{n}$$

4 Average deviation of the mean (D) - the average deviation of individual data items from the mean (d) divided by the square root of the number of data items (n)

The definition of the average deviation of the mean can be expressed by the formula:

$$D = \frac{d}{\sqrt{n}}$$

5 Variance - the sum of the squares of the deviations of the values from their mean divided by the total number of data values (n) minus 1

The definition of the variance can be expressed by the following formula:

$$s^2 = \frac{\Sigma(X_i - \overline{X})^2}{n - 1}$$

6 Standard deviation - the square root of the variance

The definition of the standard deviation can be expressed by the following formula:

$$s = \sqrt{\frac{\Sigma(X_i - \overline{X})^2}{n - 1}}$$

However, the formula commonly used because of its adaptability to the hand calculator is the following:

$$s = \sqrt{\frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n - 1}}$$ where there are n number of values.

7 Standard deviation of the mean (S) - the standard deviation of individual data items (s) divided by the square root of the number of data items (n)

The definition of the standard deviation of the mean can be expressed by the formula:

$$S = \frac{s}{\sqrt{n}}$$

8 Relative standard deviation - the standard deviation (s) expressed as a fraction of the mean, $\frac{s}{\overline{X}}$

The relative standard deviation is often expressed as a percent. It is then referred to as the coefficient of variation (V):

$$V = \frac{s}{\overline{X}} \times 100 = \%$$

The relative standard deviation is particularly helpful when comparing the precision of a number of determinations on a given substance at different levels of concentration.

B Aids in Calculation

Application of the following statements can reduce errors and amount of time spent in calculating the variance or standard deviation of a distribution.

1 Adding or subtracting a constant to or from each score in a distribution doesn't affect the variance or standard deviation of the distribution.

Thus the following formulas:

(1) $s_c^2 = s^2$

(2) $s_c = s$

where the X_i's are the values in the distribution with variance s^2 and standard deviation s, and the $X_i \pm C$'s are the values in the distribution with variance s_c^2 and standard deviation s_c.

2 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the variance of that distribution by the square of the same constant.

Thus the following formulas:

(1) $s_c^2 = C^2 s^2$

(2) $s_c^2 = \frac{s^2}{C^2}$

where the X_i's are the values in the distribution with variance s^2, and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with variance s_c^2.

3 Multiplying or dividing each score in a distribution by a constant is equivalent to multiplying or dividing the standard deviation of that distribution by the same constant.

Thus the following formulas:

(1) $s_c = Cs$

or

(2) $s_c = \frac{s}{C}$

where the X_i's are the values in the distribution with standard deviation s, and the CX_i's or the $\frac{X_i}{C}$'s are the values in the distribution with standard deviation s_c.

C Application

Consider the application of the above definitions to the previously mentioned set of data, obtained from twelve determinations for chloride in water, shown in II B, Table 1.

1 Range = 102 -98 = 4

2 Average deviation - $d = \frac{\Sigma|X_i - \bar{X}|}{n}$

n	X_i	$\|X_i - \bar{X}\|$	$n\|X_i - \bar{X}\|$
1	98	2.25	2.25
2	99	1.25	2.50
4	100	.25	1.00
3	101	.75	2.25
2	102	1.75	3.50
$\bar{X}$ = 100.25			11.50

$$d = \frac{\Sigma|X_i - \bar{X}|}{n} = \frac{11.50}{12} = .96$$

3 Average deviation of the mean -

$$D = \frac{d}{\sqrt{n}}$$

Using calculations from number 2,

$$D = \frac{d}{\sqrt{n}} = \frac{0.96}{\sqrt{12}} = \frac{0.96}{3.46} = 0.28$$

4 Variance - $s^2 = \frac{\Sigma(X_i - \bar{X})^2}{n-1}$

n	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	$n(X_i - \bar{X})^2$
1	98	-2.25	5.06	5.06
2	99	-1.25	1.56	3.12
4	100	- .25	.06	.24
3	101	+ .75	.56	1.68
2	102	+1.75	3.06	6.12
				16.22

$$s^2 = \frac{\Sigma(X_i - \bar{X})^2}{n - 1} = \frac{16.22}{11} = 1.47$$

5 Standard deviation - $s = \sqrt{\frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n - 1}}$

n	X_i	nX_i	X^2_i	nX^2_i
1	98	98	9604	9604
2	99	198	9801	19602
4	100	400	10000	40000
3	101	303	10201	30603
2	102	204	10404	20808
		1203		120617

$$s = \sqrt{\frac{120617 - \frac{1203^2}{12}}{11}} = \sqrt{\frac{120617 - 120601}{11}}$$

$$s = \sqrt{\frac{16}{11}} = 1.21$$

6 Aid in calculation

Recalling that adding or subtracting a constant to each score in the distribution doesn't affect the variance or the standard deviation of the distribution we can simplify the computations by first subtracting 100 from each score in the distribution, thus obtaining the frequency distribution shown in Table 2.

n	X_i-C	$n(X_i$-C$)$	$(X_i$-C$)^2$	$n(X_i$-C$)^2$
1	-2	-2	4	4
2	-1	-2	1	2
4	0	0	0	0
3	1	3	1	3
2	2	4	4	8
		3		17

$$s^2 = \frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n - 1}$$

$$s^2 = \frac{17 - \frac{(3)^2}{12}}{11} = \frac{16.25}{11} = 1.48$$

$$s = \sqrt{\frac{\Sigma X_i^2 - \frac{(\Sigma X_i)^2}{n}}{n - 1}} = \sqrt{1.48} = 1.22$$

7 Standard deviation of the mean -

$$S = \frac{s}{\sqrt{n}}$$

Using calculations from number 6,

$$S = \frac{s}{\sqrt{n}} = \frac{1.22}{\sqrt{12}} = \frac{1.22}{3.46} = 0.35$$

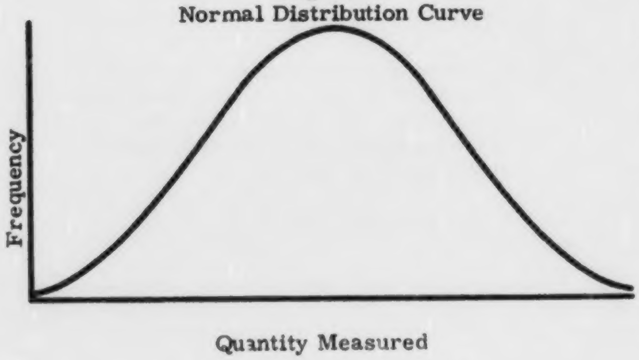
8 Relative standard deviation expressed as a percent (coefficient of variation)

$$V = \frac{s}{\overline{X}} \times 100$$

Using calculations from number 6 for $s = 1.22$ and from number 2 for $\overline{X} = 100.25$,

$$V = \frac{s}{\overline{X}} = \frac{1.22}{100.25} \times 100 = 1.21\%$$

Figure 2
Normal Distribution Curve



V INTRODUCTION TO NORMAL DISTRIBUTION CURVE

A Statistics deals with theoretical curves which are smoother than frequency polygons, obtained from experiments in real life. However, frequency distributions or frequency polygons of experimental data often approximate a mathematical function called the "normal" distribution curve. (See Figure 2)

As shown in Figure 3, the frequency polygon for the 12 determinations for chloride in water is a fairly good approximation of the normal curve. If, however, in the chloride determinations we had obtained 103 instead of 98 and 104 instead of 99 this distribution would not have been a good approximation of the normal curve, as is shown in Figure 4.

Figure 3

Comparison of Normal Curve and Frequency Polygon

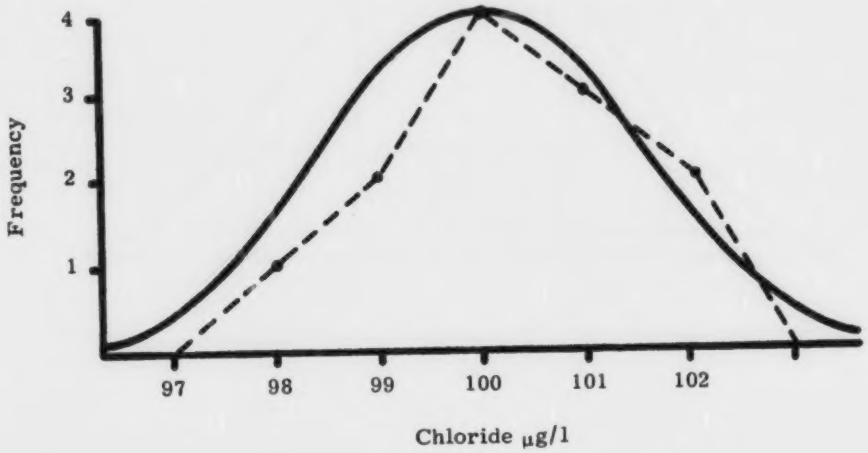
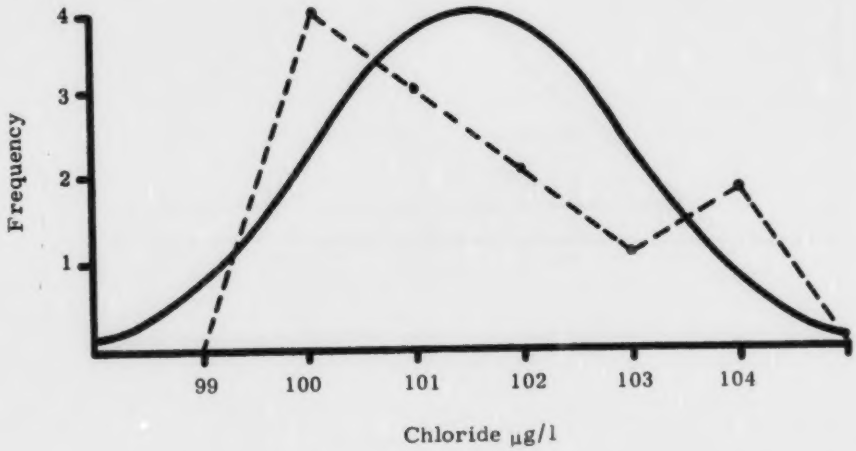


Figure 4

Comparison of Normal Curve and Frequency Polygon



B **If a frequency distribution is a good approximation of the normal curve, we can use some facts about the normal curve to give us information about the frequency distribution.**

Figure 5 shows the normal distribution in terms of the population mean μ, and the standard deviation of the population σ, and gives the percent of area under the curve between certain points.

Figure 5

Normal Distribution Curve

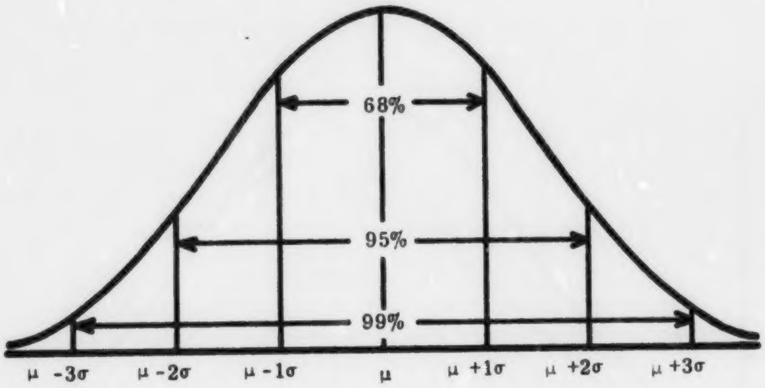
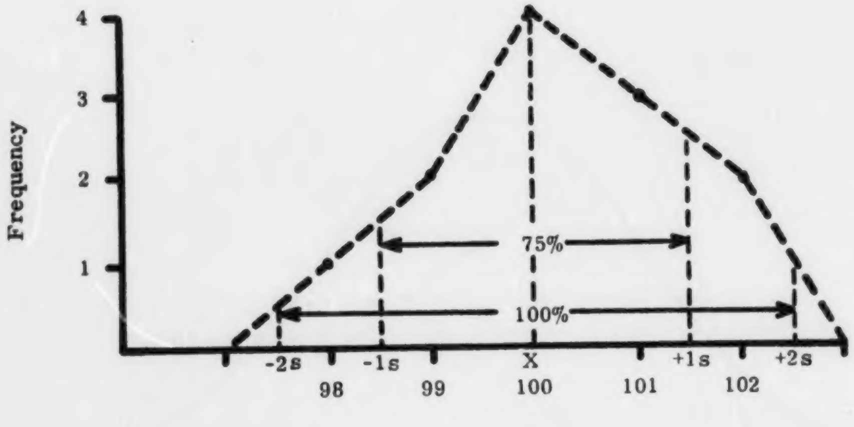


Figure 6

Frequency Distribution Polygon



Chloride μg/l

We may check the distribution of sample data to see if it is a "normal" distribution in the following manner. Substitute the value of the sample mean ($\overline{X}$) for the value of the midline and substitute the value of the sample standard deviation (s) for the limits of the value spans where we might expect certain percentages of the data items to occur. Then we can check the number of data items which actually do occur within these value spans.

Figure 6 demonstrates this application using the chloride data values from Table 1. The data values are marked on the horizontal line and the frequency of the occurrence of each value is marked on the vertical. The midline of the distribution is marked at the value of the sample mean ($\overline{X}$ = 100, See III C 3). The value of the sample standard deviation (s = 1.21, See IV C 5) is used to mark value areas under the curve where different percentages of data values will probably occur. Thus, for the area $\overline{X} \pm 1s$, $\overline{X} - 1s = 98.79$ and $\overline{X} + 1\ s = 101.21$. Therefore, according to the normal distribution curve shown in Figure 5, we might expect about 68% of the data items to have values between 99 and 101. (The values are rounded to whole numbers since the data values are thus recorded).

Consulting Table 1, we find that 75% or 9 of the 12 data items have values in this range. This percentage is shown in Figure 6 by the frequency polygon for the data shown earlier in Figure 3.

Likewise assuming a normal distribution, we would expect 95% of the observations to lie within $\pm 2\sigma$'s from the population mean. In fact, 100% of the observations were within $\pm$ 2 s's from the sample mean.

In both cases the observed percentages are reasonably close to the expected percentages. Other tests exist for determining whether or not a frequency distribution might reasonably be assumed to approximate the normal distribution.

It would be good to become as familiar as possible with the normal distribution since an underlying normal distribution is assumed for many statistical tests of hypothesis.

REFERENCES

1 Bennett, C.A. and Franklin, N.L. Statistical Analysis in Chemistry and the Chemical Industry. John Wiley & Sons, Inc., New York. 1954.

2 Crow, E.L., Davis, F.A., and Maxfield, M.W. Statistics Manual. Dover Publications, Inc., New York. 1960.

3 Dixon, W.J. and Massey, F.J. Introduction to Statistical Analysis. McGraw-Hill Book Co., Inc., New York. 1957.

4 Ostle, B. Statistics in Research. The Iowa State University Press, Iowa. 1963.

5 Youdon, W.J. Statistical Methods for Chemists. John Wiley & Sons, Inc., New York. 1951.

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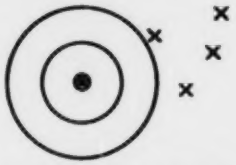
Descriptors: Graphic Methods, Quality Control, Statistical Methods, Statistics

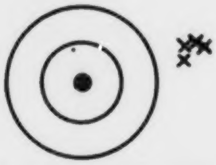
ACCURACY-PRECISION-ERROR

I INTRODUCTION

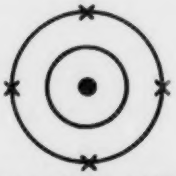
An analytical method is subject to errors. These errors may affect the accuracy of the method because they introduce bias into the results. There are other types of errors which affect the precision of the method because they produce random fluctuations in the data. The most desirable situation for the analyst is shown in the diagram in Figure 1 where the results are both precise and accurate.



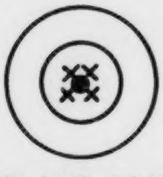
IMPRECISE AND INACCURATE



PRECISE BUT INACCURATE



ACCURATE BUT IMPRECISE



PRECISE AND ACCURATE

Figure 1. PRECISION AND ACCURACY

A Accuracy

For results to be accurate, the analysis used must give values close to the true value.(See Figure 1)

B Precision

Precision is the degree of agreement among results obtained by repeated measurements on a single sample under a given set of conditions. It is a measure of the degree to which results "check." (See Figure 1)

C Note

It is possible to have precision without accuracy. (See Figure 1)

II DETERMINATE ERROR AND ACCURACY

A determinate error is one which contributes a constant error or bias to results, causing them to be inaccurate. This constant error makes it possible for results to agree with each other (be precise) and still be inaccurate.(See Figure 1)

Determinate errors have "assignable" causes which can usually be identified and either eliminated or controlled. (The terms "determinate" error, "assignable" error, and "systematic" error are synonymous).

A Sources of Determinate Error

1 Method error

Method errors are those that are inherent in the procedure. These are very serious and the hardest to detect and correct. The most common method error is the presence of interferences in the sample. Other examples would be precipitation of substances other than the desired material, partial solubility of precipitates, and entrainment as in a solvent extraction procedure.

2 Personal errors

Personal errors are attributable to individual mistakes which are consistently made by an analyst. These errors are the result of consistent carelessness, lack of knowledge or personal bias. Examples are errors in calculations, use of contaminated reagents, non-representative sampling, or poor calibration of standards and instruments.

3 Instrumental errors

Instrumental errors are those which are caused by an analytical instrument or by the effects of the environment acting on the instrument. Moisture in a G. C. column, improper wavelength markings on a spectrophotometer or incorrect scoring on a buret would be examples.

B Effects of Determinate Error

1 Additive

An additive determinate error is one which has a constant value regardless of the amount of analytically sought constituent present in the sample. (See Figure 2)

2 Proportional

A proportional determinate error changes value according to the amount of analytically sought constituent in the sample. (See Figure 3)

III DETECTION OF DETERMINATE ERROR

A Spiked Samples

1 Samples which can be determined before and after the addition of a known constituent (in the concentration range of interest) provide a way to detect determinate errors. Spiked samples should be representative and resemble actual conditions as closely as possible. The quantitation of bias can then be obtained with the following measures.

a Mean error - the difference between the mean of the data and the true result.

b Relative error - the mean error of a set of data expressed as a percentage of the true result.

EXAMPLE: An analyst determines the nitrate content of the effluent from his sewage treatment plant to be 0.5 mg/l. He then adds 1 mg/l of standard nitrate solution to the sample. Table 1 shows the replicate results obtained on the spiked sample, and calculation of both mean and relative errors.

Table 1

Sample: Effluent
Determination: Nitrate (Modified Brucine)

X_i
1.55 mg/l
1.58
1.60
1.47
1.35

1 Calculation of mean error

$$\bar{X} = \frac{7.55}{5} = 1.51 \text{ mg/l}$$

Mean error = +0.01 mg/l

2 Calculation of relative error

$$\text{Relative Error} = \frac{0.01 \times 100}{1.50} = 0.7$$

2 Control charts

Trends and shifts on control charts may also indicate determinant error. Using spiked samples, the standard deviation is calculated and control limits (usually ± 3 standard deviations) for the analysis are set (see Figure 4. For further discussion of control limits, see reference 3, p. 62.

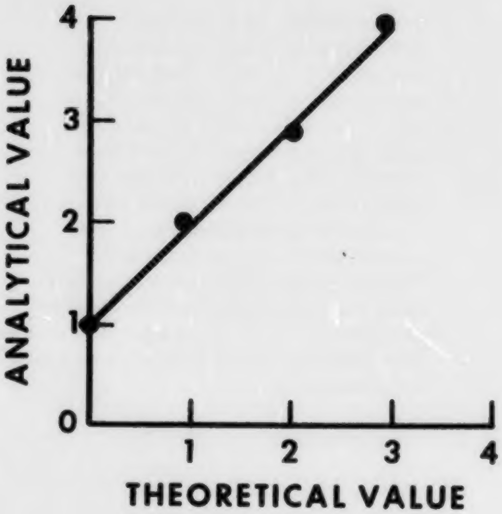


Figure 2. ADDITIVE ERROR

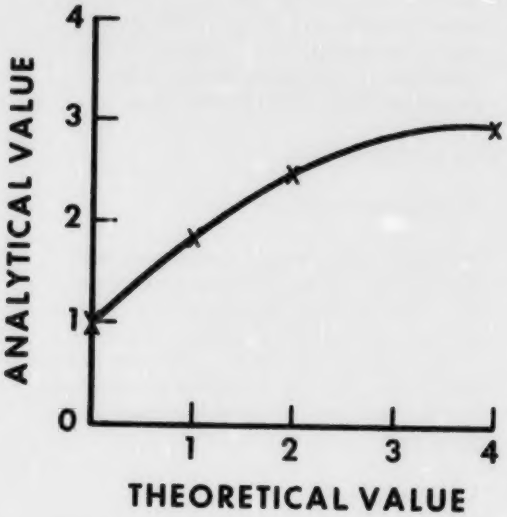


Figure 3. PROPORTIONAL ERROR

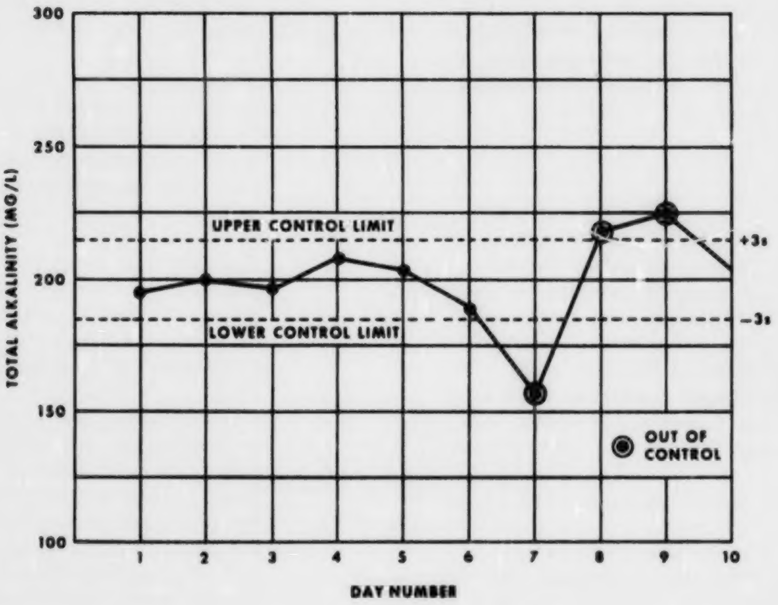


Figure 4. CONTROL CHART

3 In some cases, it is impossible to spike a sample so that it resembles actual conditions (e.g., BOD and pesticide samples). Youden (11) provides excellent techniques for detecting bias in this situation.

B Unknown Samples

1 Independent method

Analysis of a sample for a desired constituent by two or more methods that are entirely different in principle (gravimetric and volumetric) may aid in the estimation of determinate error. However, another reliable method may not be available or may be laborious to perform.

2 Control charts

It is possible to plot a control chart (Figure 4) even when it is not possible to spike a sample. One can use as a reference value an average of a series of replicate determinations performed on a composite check sample. Such a sample must be preserved or stabilized in such a way that the concentration of the constituent being measured will not change from day to day (see reference 1).

3 Aliquoting

If the determinate error is additive, the magnitude may be estimated by plotting the measured quantity versus a range of sample volumes or sample weights. If the error has a constant value regardless of the amount of analytically sought constituent, then a straight line fitted to the points will not go through the origin. (See Figure 5)

C Youden's Graphical Technique (10, 11, 12)

Dr. W. J. Youden has devised an approach to test for determinant errors with a minimum of effort on the part of the analyst and his laboratory. Samples used may be of known (spiked) or unknown composition.

1 Technique

Two different test samples (X and Y) are prepared and distributed for analysis to as many individuals or laboratories as possible. Each participant is asked to perform only one determination on each sample (NOTE: It is important that the samples be relatively similar in concentration of the constituent being measured). Table 2 shows the results on two such samples analyzed for percent potassium by 14 different laboratories. The mean for each sample has been calculated.

2 Interpretation - Figure 6

The vertical line drawn on the graph represents the mean $(\overline{X})$ of all the results obtained on Sample X; the horizontal line was drawn through the mean $(\overline{Y})$ of all the results obtained on sample Y. Each pair of laboratory results can then be plotted as a point on the graph (marked X. 1 etc.)

If the ratio of the bias (error) to standard deviation is close to zero for the determinations submitted by the participants, then one would expect the distribution of the paired values (or points) to be close to equal among the four quadrants. The fact that the majority of the points fall in the (+, +) and (-, -) quadrants indicates that the results have been influenced by some source of bias or determinate error.

Furthermore, one can even learn something about a participant's precision from the graph. If all participants had perfect precision (no indeterminate error), then all the paired points would fall on a 45° line passing through the origin. Consequently the distance from such a 45° line of each participant's point provides an indication of that participant's precision.

3 The Youden method is also discussed in this manual in the section on Interlaboratory Quality Control Studies. A quantitative treatment of this subject can be found in references 11 and 12.

Table 2

Laboratory	Sample X	Sample Y
1	9.74%	8.50%
2	9.92	8.28
3	9.98	8.84
4	9.99	8.24
5	10.00	8.73
6	10.11	8.54
7	10.12	8.64
8	10.14	8.82
9	10.19	9.04
10	10.23	8.93
11	10.25	8.97
12	10.29	8.80
13	10.55	9.21
14	10.62	8.95

$\overline{X} = 10.15$ $\overline{Y} = 8.75$

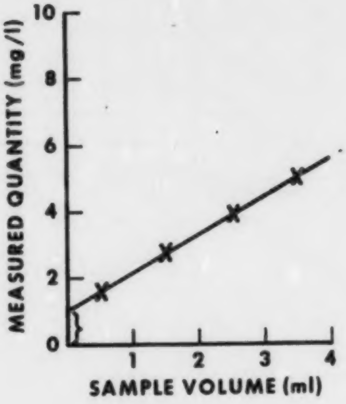


Figure 5

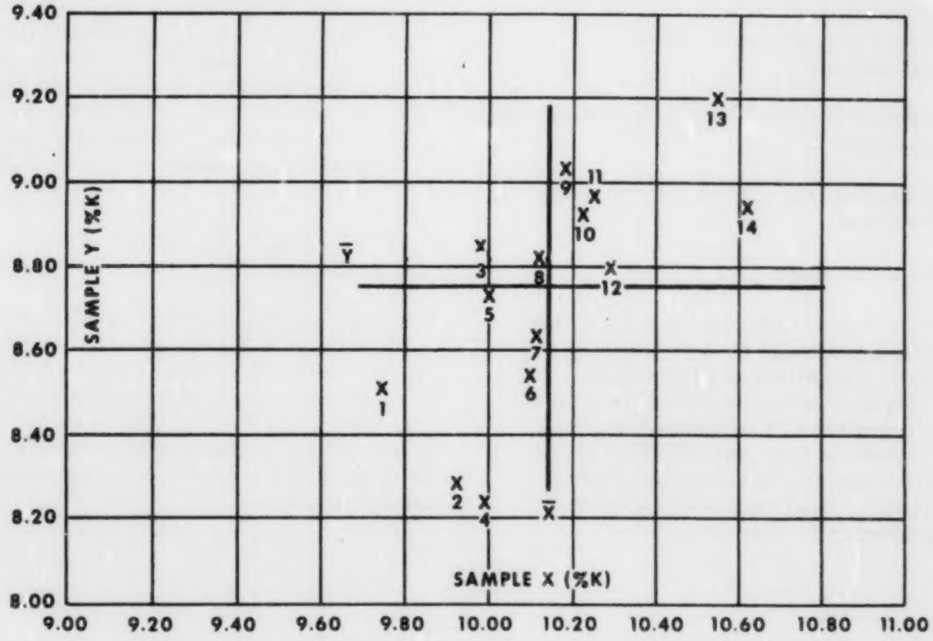


Figure 6. YOUDEN'S GRAPHICAL TECHNIQUE

IV ELIMINATION OF DETERMINATE ERROR

Various approaches can be used to eliminate the source of determinate error. The approach used depends upon whether the source is personal, method, or instrumental.

A Personal

Great care must be used to avoid producing an unconfident attitude in a technician. It is undesirable for the analyst to feel he is being "policed."

B Method and Instrumental

1 Blanks

Blanks can be used to correct for interferences from reagents, sample color, etc.

2 Correction factors

Examples of correction factors used in environmental analyses to eliminate determinate errors are the following:

a Recovery factors in organic extractions

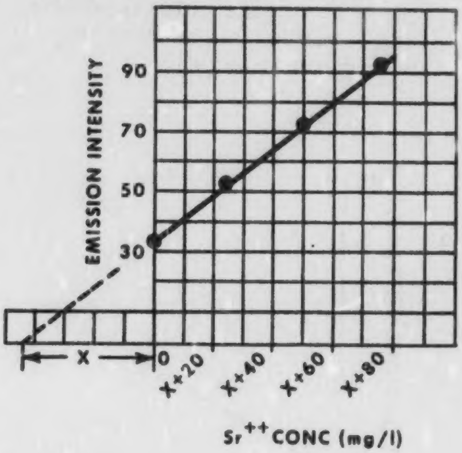
b Chemical yield values in gravimetric analyses

c Counting efficiencies for radiation counters

3 Standard addition

Sample interferences producing determinate errors can be overcome by adding equal amounts of unknown sample to a series of standards. The concentration of the unknown can then be determined graphically from a plot of the measured quantity (absorption, emission, etc.) versus the standard concentration. (See Figure 7)

Figure 7. METHOD OF STANDARD ADDITION GRAPHICAL METHOD OF COMPUTING Sr CONCENTRATION



NOTE: The X value appearing along the abscissa (i.e., X + 20, X + 40, etc.) refers to the unknown added to the strontium standard

4 Standard compensation

Another approach is to prepare the standard so that its composition resembles that of the sample as closely as possible. The objective of the approach is exactly the same as that of standard addition - to compensate for the presence of interfering substances in the unknown.

V INDETERMINATE ERROR AND PRECISION

Even when all determinate errors are eliminated, every replicate analysis will not give the same value. Such variation in results is due to indeterminate error, also known as random, chance or uncontrollable error. Indeterminate error affects the precision or agreement among results.

A Sources of Indeterminate Error

Indeterminate errors are due to unassignable or "chance causes." Examples are inadvertent contamination of sample or glassware, variation in reagent additions, or variations in instrument response (see Figure 8).

B Effects of Indeterminate Error

Since the causes of these errors are random, the effects are also random. Fortunately, these random variations conform to the "Laws of Chance" so statistical measures of precision can be used to quantitate indeterminate errors.

VI DETECTION OF INDETERMINATE ERROR

A measure of the degree of agreement among results can be obtained by analyzing a single sample repeatedly under a given set of conditions.

A Range

The range of the replicate results (difference between the lowest and the highest value) provides a measure of indeterminate variations.

B Standard Deviation

An estimation of indeterminate error can be obtained through a calculation of the standard deviation. The following formulas should be applied to random data which follows a normal distribution. Normality can be checked by ranking and plotting the data on normal probability paper; it should fall on a straight line (see Figure 9). Any values which do not fall close to the straight line (those values encircled) should be rejected in the calculation of standard deviation. Other statistical tests (8) can be used to objectively evaluate the rejection of outliers. The value obtained for standard deviation for a particular method may vary with the analyst, the concentration range of the constituent, and the composition of the sample analyzed. The confidence of the estimate is increased as the number of results (n) used to compute the standard deviation is increased.

1 Replicate results on the same sample:

$$s = \sqrt{\frac{\Sigma(X_i - \overline{X})^2}{n-1}} \qquad (1)$$

X_i = value of single result

$\overline{X}$ = average (mean) of results on same sample

n = number of results

Example: Table 3 contains a set of 5-day BOD results obtained on a synthetic sample containing 150 mg/l of glucose and 150 mg/l glutamic acid. (Note: A 1% dilution was used in the actual test). The results are those submitted by laboratories participating in a colaborative study. Calculate the standard deviation of these results.

$\overline{X}$ = 192 mg/l

n = 36.0

$\Sigma(X_i - \overline{X})^2$ = 58,200

$$s = \sqrt{\frac{58,200}{35}}$$

s = 41 mg/l

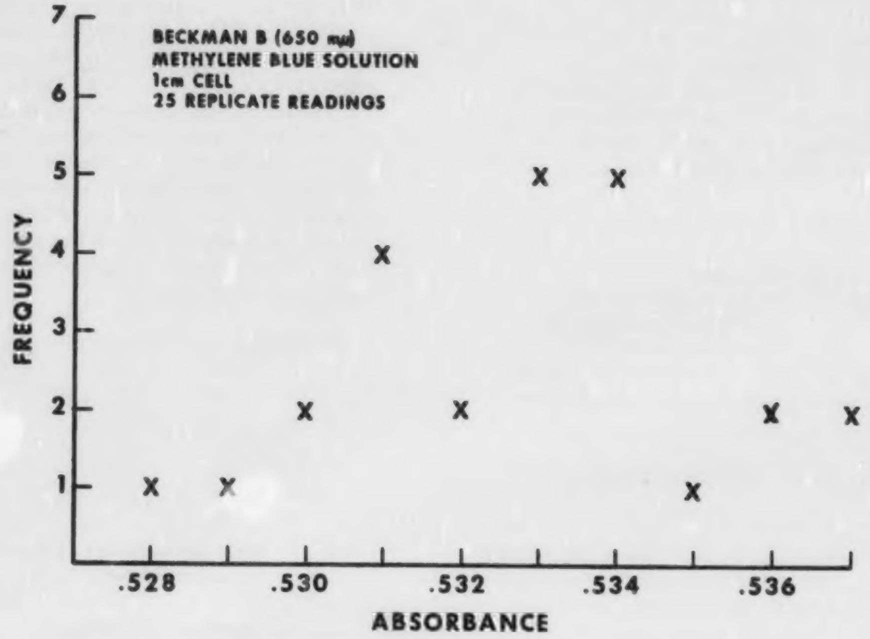


Figure 8. VARIATIONS IN SPECTROPHOTOMETER READINGS

FIGURE 9 NORMAL PROBABILITY CURVE

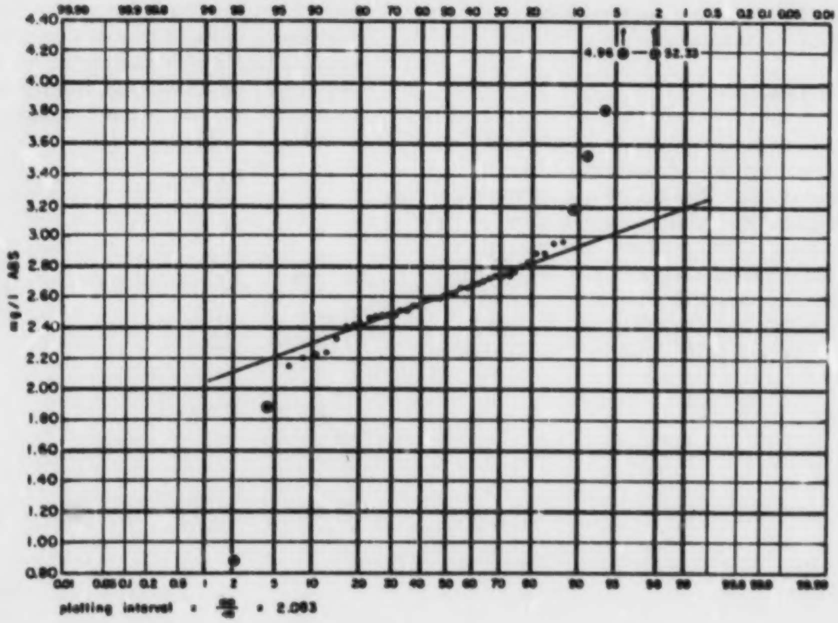


Table 3

SAMPLE: 150 mg/l glucose + 150 mg/l glutamic acid (1% dilution)
DETERMINATION: 5-day Biochemical Oxygen Demand

X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$	X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
100 mg/l	-92 mg/l	8464 $(mg/l)^2$	198 mg/l	6 mg/l	36 $(mg/l)^2$
117	-75	5625	199	7	49
125	-67	4489	200	8	64
132	-60	3600	200	8	64
142	-50	2500	204	12	144
147	-45	2025	210	18	326
153	-39	1521	211	19	361
160	-32	1024	212	20	400
165	-27	729	215	23	529
165	-27	729	223	31	961
167	-25	625	224	32	1024
173	-19	361	227	35	1225
178	-14	196	229	37	1369
189	- 3	9	238	46	2116
190	- 2	4	247	55	3025
196	4	16	250	58	3364
196	4	16	259	67	4489
197	5	25	274	82	6724

*Data taken from Water, Oxygen Demand Report (July, 1960), Analytical Reference Service, Training Program, R. A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

Table 4*

SAMPLE: Aqueous
CONCENTRATION RANGE: 30 - 70 mg/l
DETERMINATION: Phosphate (Lucerna, Conde, and Prat Method)

Sample	Mg/l P	d	d^2	Sample	Mg/l P	d	d^2
A	51	2	4	L	54	2	4
	53				56		
B	39	0	0	M	38	1	1
	39				37		
C	53	1	1	N	52	0	0
	54				52		
D	47	0	0	O	58	0	0
	47				58		
E	50	1	1	P	54	0	0
	51				54		
F	48	1	1	Q	54	0	0
	47				54		
G	50	0	0	R	48	0	0
	50				48		
H	47	0	0	S	52	1	1
	47				51		
I	42	0	0	T	53	0	0
	42				53		
J	50	1	1	U	46	1	1
	51				47		
K	59	1	1	V	42	0	0
	60				42		

*Data obtained from Frank Schickner, Proctor and Gamble Company.

2 Duplicate results on different samples

In a laboratory where duplicates are routinely run, it would be simple to use the following formula for evaluation of standard deviation.

$$s = \sqrt{\frac{\Sigma(d^2)}{2k}} \quad \text{(See reference 5, page 654)} \qquad (2)$$

d = difference between duplicates

k = number of samples

Example: Table 4 contains a set of phosphate results obtained on aqueous samples in the range 100-200 mg/l. Calculate the standard deviation of these results.

$\Sigma(d^2) = 16$

$k = 22$

$s = \sqrt{\frac{16}{44}}$

$s = .61$ mg/l

3 Duplicate and triplicate results on different samples

$$s = \sqrt{\frac{\Sigma(X_i - \bar{X})^2}{n - k}} \quad \text{(See reference 7, page 73)} \qquad (3)$$

Note: $\bar{X}$ = average of results on the same sample

Example: Table 5 contains a set of % nitrogen results obtained on unknown organic compounds.

Calculate the standard deviation.

$\Sigma(X_i - \bar{X})^2 = .2401$

$n - k = 22$

$s = \sqrt{\frac{.2401}{22}}$

$s = .104\%$

4 Use of range to estimate standard deviation

For a small number of replicates ($n < 10$), the range can be used to estimate the standard deviation (See Table 6).

$$\frac{R}{d_N} = s \qquad (4)$$

Example: Table 7 contains a set of replicate nitrate results. Calculate the standard deviation of these results.

a Use of formula (1)

$\bar{X} = 0.72$ mg/l

$n = 5$

$\Sigma(X_i - \bar{X})^2 = .0134$

$s = \sqrt{\frac{.0134}{4}}$

$s = .058$ mg/l

b Use of formula (4)

$R = .14$ mg/l

$d_N = 2.33$

$s = \frac{.14 \text{ mg/l}}{2.33}$

$s = .060$ mg/l

C Coefficient of Variation

An estimation of indeterminate error can also be made by calculating the coefficient of variation.

$$V = \frac{s}{\bar{X}} \times 100$$

By comparing the standard deviation (dispersion) to the average or mean value (central tendency) in a set of data and expressing this relative standard deviation as a percentage, the analyst has a meaningful interpretation of the degree of dispersion present. Indeter-

Table 5*

SAMPLE: Unknown Organic Compounds
DETERMINATION: % Nitrogen (Kjeldahl)

CONCENTRATION RANGE: 10% - 20%

Sample	%N	$\bar{X}$	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
A	16.48	16.47	.01	.0001
	16.46		.01	.0001
B	16.50	16.50	0	0
	16.49		.01	.0001
C	16.72	16.65	.07	.0049
	16.57		.08	.0064
D	17.52	17.58	.06	.0036
	17.60		.02	.0004
	17.63		.05	.0025
E	16.31	16.31	0	0
	16.30		.01	.0001
F	16.40	16.35	.05	.0025
	16.31		.04	.0016
	16.35		0	0
G	17.56	17.55	.01	.0001
	17.54		.01	.0001
H	14.96	14.81	.15	.0225
	14.66		.15	.0225
I	19.15	19.02	.13	.0169
	18.89		.13	.0169
J	13.93	13.75	.18	.0324
	13.56		.19	.0361
K	10.34	10.27	.07	.0049
	10.19		.08	.0064
L	17.16	17.15	.01	.0001
	17.13		.02	.0004
M	15.01	15.03	.02	.0004
	15.05		.03	.0004
N	12.44	12.62	.18	.0324
	12.70		.08	.0064
	12.73		.11	.0121
O	14.37	14.37	0	0
	14.36		.01	.0001
P	11.85	11.85	0	0
	11.85		0	0
Q	14.79	14.73	.06	.0036
	14.70		.03	.0009
	14.70		.03	.0009
R	17.19	17.17	.02	.0004
	17.14		.03	.0009

*Data obtained from Frank Schickner, Proctor and Gamble Company.

Table 6*

FACTORS USED TO ESTIMATE THE STANDARD DEVIATION FROM RANGE

Size of Sample (n)	d_N	$\frac{1}{d_N}$
2	1.13	.887
3	1.69	.591
4	2.06	.486
5	2.33	.430
6	2.53	.395
7	2.70	.370
8	2.85	.351
9	2.97	.337
10	3.08	.325

*Natrella, Experimental Statistics(6), pp. 2-6.

Table 7

SAMPLE: Ohio River Water

DETERMINATION: Nitrate (Modified Brucine)

X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
0.65 mg/lN	-0.07	.0049
0.68	-0.04	.0016
0.70	-0.02	.0004
0.76	+0.04	.0016
0.79	+0.07	.0049

minate error would be the cause of undue dispersion.

Example: Table 3

$s = 41$ mg/l

$\overline{X} = 192$ mg/l

$V = \frac{41}{192} \times 100$

$V = 21\%$

D An indication of an individual's analytical precision can be obtained by his participation in an interlaboratory study. In this case, the analyst performs only one determination on each of two samples. Using Youden's Graphical Technique as an indication of an individual's precision was discussed previously in this outline in III C. Also see the outline on "Interlaboratory Quality Control Studies."

REFERENCES

1 Accuracy in Clinical Chemistry. Dade Reagents, Inc., Miami, Florida.

2 Allan, Douglas H. Statistical Quality Control. Reinhold Publishing Corp., New York. 1959.

3 American Society for Testing Materials ASTM Manual on Quality Control of Materials. Special Technical Publication 15-C. 1951.

4 Bauer, E.L. A Statistical Manual for Chemists. Academic Press, New York. 1960.

5 Bennett, Carl A. and Franklin, Norman L. Statistical Analysis in Chemistry and the Chemical Industry. John Wiley & Sons, Inc., New York. 1954.

6 Chase and Rabinocvitz. Principles of Radioisotope Methodology. Minneapolis Burgess Publishing Company. 1964.

7 Mickley, Harold S., Sherwood, Thomas K. and Reed, Charles E. Applied Mathematics in Chemical Engineering. McGraw-Hill Book Company, New York. 1957.

8 Natrella, M.G. Experimental Statistics, National Bureau of Standards Handbook 91. U.S. Dept. of Commerce. 1963.

9 Schickner, Frank A. Personal Communication. The Procter & Gamble Company, Miami Valley Laboratories, Research & Development Department, P.O. Box 39175, Cincinnati, OH 45239.

10 Youden, W.J. The Collaborative Test. JAOAC 46:55-62. January 1963.

11 Youden, W.J. The Sample, The Procedure, and the Laboratory. Anal. Chem. 32:23-37A. Dec. 1960.

12 Youden, W.V. Statistical Techniques for Collaborative Tests. Association of Official Analytical Chemists, Box 540, Benjamin Franklin Station, Washington, DC . 1967.

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Descriptors: Accuracy, Data Collections, Data Processing, Error Analysis, Errors, Graphic Methods, Measurement, Monitoring, Precision, Quality Control

INTERLABORATORY QUALITY CONTROL STUDIES

I DEFINITION OF INTERLABORATORY STUDY IN EPA

A It is a between-laboratory evaluation of exact physical, chemical or biological methods which yield a measurement of desired properties.

B It is not an interlaboratory evaluation of materials, reagents, or different test conditions. It is not an initial study of a method, nor a study to develop methods.

II PURPOSES OF INTERLABORATORY STUDY

A Selection of an analytical method.

B Evaluation of an analytical method.

C Evaluation of laboratory and analyst performance.

III PREPARATION FOR INTERLABORATORY STUDY

A Laboratories are in control. Between-replicate deviation will then be very small and uniform in all laboratories.

B Ruggedness testing of a method has been completed by the method developer, or by a single qualified laboratory. Effects of small changes in time, temperature, pH, reagent and sample volume measurements, etc., are known and corrected for.(1)

C All laboratories and analysts are familiar with the method to be tested. Any special equipment or reagents required are known and available., If all analysts are not acquainted with the method, a simple preliminary study is undertaken to accomplish this.(2)

IV INSTRUCTIONS FOR THE STUDY

A Exact method write-ups are provided to each analyst. The technical description of an analytical method is difficult. The language and organization must be complete, yet simple and unambiguous. These characteristics are often conflicting.

Requirements in a method may seem trivial. Yet if these are not recognized and controlled, the entire study can be useless.

B Explicit directions for sample preservation, sample make-up, time limitations, sequence of analyses, etc. , are provided.

C Advance notice of tests is given so that laboratories can integrate the test into their program and realistic deadlines can be established. Once established, deadlines for agreement to participate, for completion of analyses and for reporting are followed closely.

V THE TEST SAMPLES

A The sample is carefully designed to reflect the concentrations found in natural waters and yet be within the best portion of the concentration range for the method.

B Since precision of almost all methods varies with concentration, a comprehensive study includes several levels of concentration.

C Since accuracy of a method is very important, exactly known levels of constituents are added both to distilled and natural waters for testing. Stabilized natural waters are not used as samples.

VI SAMPLE FORM AND CONTAINER

A In the EPA Interlaboratory Program, samples are prepared as concentrates for final dilution at the testing laboratory. Use of concentrates has several advantages. This greatly reduces space requirements and thereby greatly reduces cost of mailing. With use of sealed glass ampules, preservation of concentrates is easily obtained through steam sterilization. Chemical preservatives can be used at fairly high levels in the concentrate and can be removed as interferences by the dilution of the concentrate to final sample volume. Theoretically, a well designed sample properly sealed in glass is stable indefinitely.

B Samples can be prepared as dilute simulated or natural water samples to be analyzed, as is. These samples have an advantage in that they reach the analyst exactly as a routine sample. There is no error in making a dilution and this source of variance in the method is removed.

Some disadvantages of such samples are: the serious logistics problem from storage if a large number of samples is involved; a relatively high cost of mailing; and a limited choice of preservation methods because of the size of containers, the probable use of plastic containers, and only limited testing of water types.

VII INTERLABORATORY STUDY DESIGN

A Sample concentrates are prepared in similar yet different concentrations at each of several levels of analysis.

B True values are calculated from the amount of a constituent added, and are used to measure the accuracy of the study method.

C Multiple constituents are prepared in a sample by related groups of analyses.

For example, a mineral and physical sample is analyzed for pH, alkalinity/acidity, specific conductance, total hardness, sulfate, chloride, fluoride, solids, calcium, magnesium, sodium and potassium. A nutrient sample is analyzed for ammonia nitrogen, nitrate nitrogen, Kjeldahl nitrogen, orthophosphate and total phosphorus.

D The analyst dilutes separate aliquots to volume with distilled water and with natural water of his choice.

E Single analyses are made for each parameter. Replicates are of limited value in evaluating a method for routine analyses because of data handling problems and failure to detect significant differences by replication.

F Recoveries are compared for distilled and for natural waters. Recovery from distilled water should indicate method bias. Differences in recoveries from distilled and natural waters should indicate interference by natural water samples.

VIII DATA EVALUATION AND REPORTING

A Rejection of Outliers-Accuracy.

The T-Test is applied to all data using the standard deviation of all data and compared with a T value at the 99% confidence interval. Accuracy is calculated after rejection of this data.

B Precision Measures

A statistical summary is developed for computerized treatment of data as suggested by Larsen(3). Method precision is reported as standard deviation, 95% confidence interval, and coefficient of variation. Method precision is reported for each sample since it varies with concentration.

C Graphic Display

Methods are evaluated using two-sample charts described by Youden(4). The values reported by an analyst for a sample pair became the x and y coordinates for a single data point on the chart. Data form ellipses around the true value because of systematic error. Random error is shown as the perpendicular distance from the 45° slope. General scatter indicates poor method precision. Bias is shown by movement of the ellipse away from the true values. Outliers are visible at some distance from the major grouping of data. Examples shown on the following pages are:

Figure 1 - Outliers,
Figure 2 - Limited precision and limited accuracy,
Figure 3 - Negative bias,
Figure 4 - Positive bias,
Figure 5 - Systematic error,
Figure 6 - Good precision and accuracy.

IX INTERLABORATORY STUDY REPORT (5, 6)

A Reports are slanted toward audience.

B All data are coded by laboratory and analyst.

C Glossary of terms is necessary.

D Computer use increases statistical capabilities tremendously. There is a danger of "over-evaluation".

E Increased efficiency in reporting data is achieved by using the computer print-out directly in a report. See Figures 7 and 8.

REFERENCES

1 Youden, W. J. 1961. Experimental Design and ASTM Committees. Materials Research & Standards, 1(11):862-867.

2 Preliminary Study, Nutrient Analyses, September 1969, Analytical Quality Control Laboratory, FWPCA, Cincinnati, Ohio.

3 Larson, Kenneth E., The Summarization of Data, J. of Quality Technology, Vol. 1, No. 1, Jan. 1969.

4 Youden, W. J. 1967. Statistical Techniques for Collaborative Tests. The Association of Official Analytical Chemists, Washington, D.C.

5 Winter, J. A. and Midgett, M. R. 1969. FWPCA Method Study 1, Mineral and Physical Analyses, Analytical Quality Control Laboratory, FWPCA, Cincinnati, Ohio.

6 Winter, J. A. and Midgett, M. R. 1970. Method Study 2, Nutrient Analyses, Manual Method, EMSL, USEPA, Cincinnati, Ohio.

This outline was prepared by J. A. Winter, Chief, Method & Performance Evaluation, EMSL, USEPA, Cincinnati, Ohio 45268.

Descriptors: Laboratory Tests, Quality Control, Statistical Methods, Testing Testing Procedures. Error Analysis. Graphic Methods

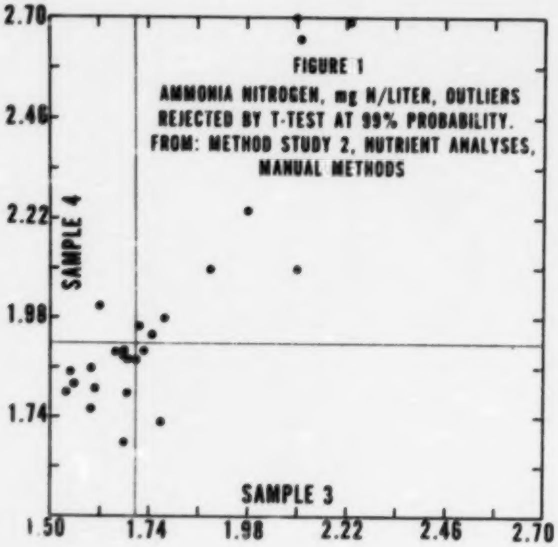


FIGURE 1

AMMONIA NITROGEN, mg N/LITER, OUTLIERS REJECTED BY T-TEST AT 99% PROBABILITY. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

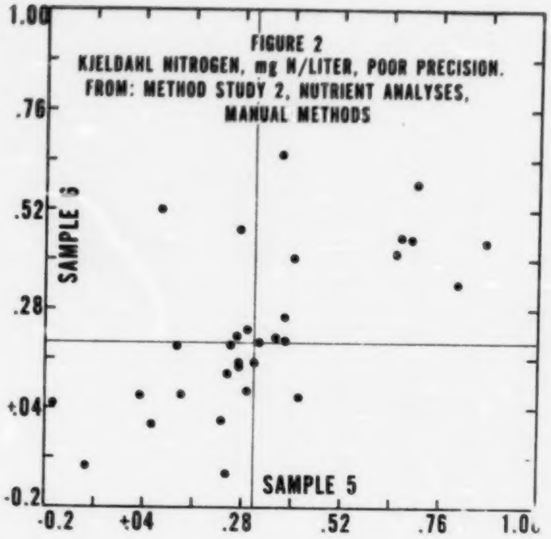


FIGURE 2

KJELDAHL NITROGEN, mg N/LITER, POOR PRECISION. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

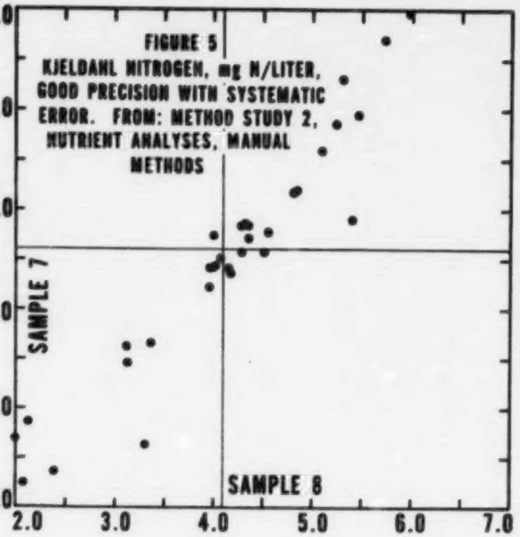


FIGURE 5

KJELDAHL NITROGEN, mg N/LITER, GOOD PRECISION WITH SYSTEMATIC ERROR. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

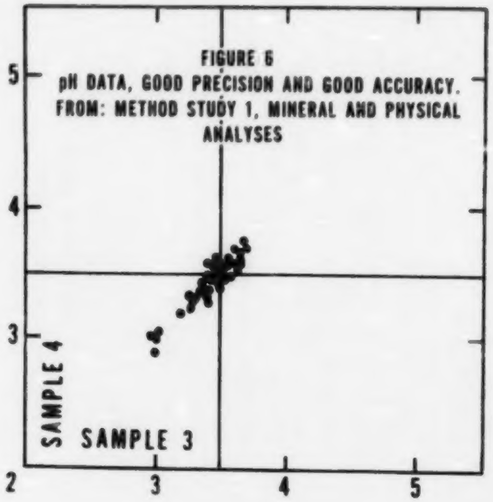


FIGURE 6

pH DATA, GOOD PRECISION AND GOOD ACCURACY. FROM: METHOD STUDY 1, MINERAL AND PHYSICAL ANALYSES

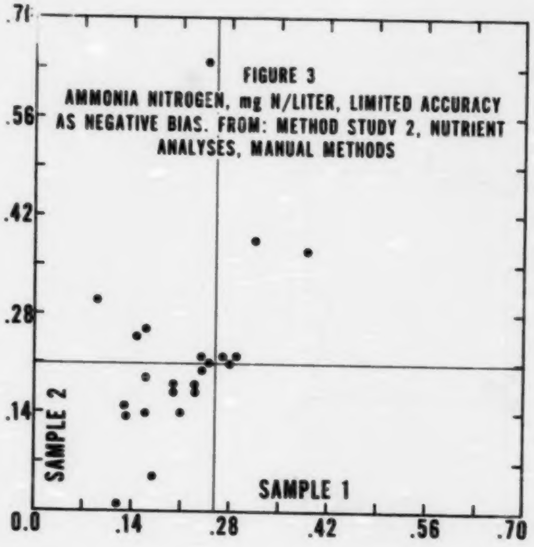


FIGURE 3
AMMONIA NITROGEN, mg N/LITER, LIMITED ACCURACY AS NEGATIVE BIAS. FROM: METHOD STUDY 2, NUTRIENT ANALYSES, MANUAL METHODS

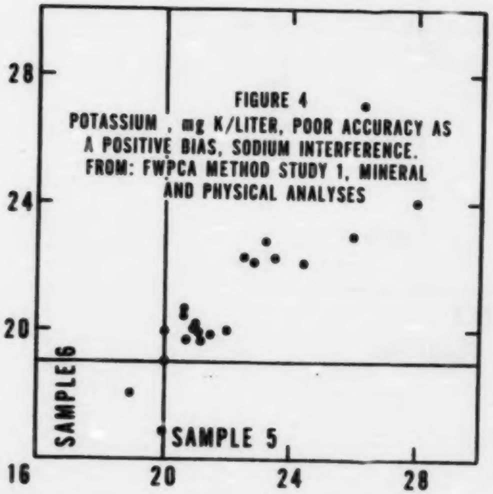
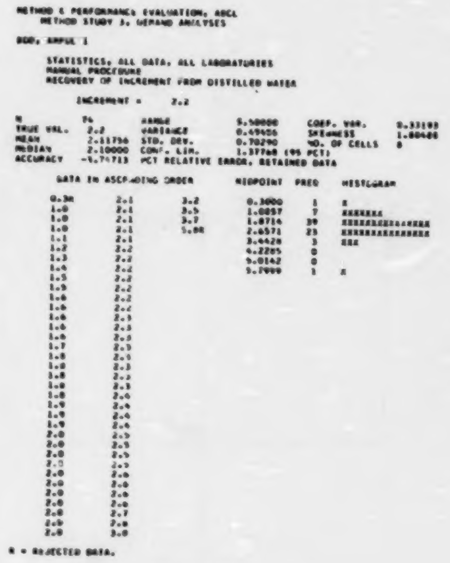


FIGURE 4
POTASSIUM , mg K/LITER, POOR ACCURACY AS A POSITIVE BIAS, SODIUM INTERFERENCE. FROM: FWPCA METHOD STUDY 1, MINERAL AND PHYSICAL ANALYSES



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METHOD & PERFORMANCE EVALUATION, AQCL
    METHOD STUDY 3, DEMAND ANALYSES

BOD, AMPUL 1

     STATISTICS, ALL DATA, ALL LABORATORIES
     MANUAL PROCEDURE
     RECOVERY OF INCREMENT FROM DISTILLED WATER

          INCREMENT =     2.2

N             74        RANGE          5.50000    COEF. VAR.      0.33193
TRUE VAL.    2.2        VARIANCE       0.49406    SKEWNESS        1.80488
MEAN         2.11756    STD. DEV.      0.70290    NO. OF CELLS    8
MEDIAN       2.10000    CONF. LIM.     1.37768 (95 PCT)
ACCURACY    -3.74713    PCT RELATIVE ERROR, RETAINED DATA

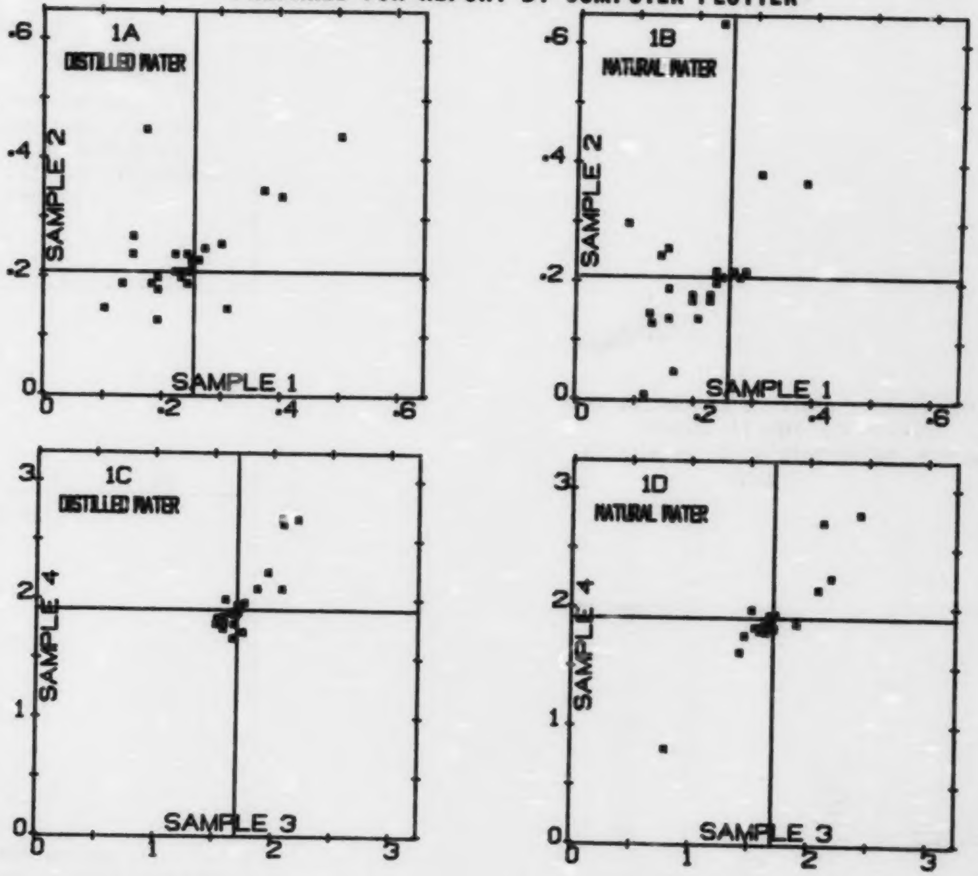
     DATA IN ASCENDING ORDER          MIDPOINT  FREQ    HISTOGRAM

     0.3R       2.1       3.2          0.3000      1    X
     1.0        2.1       3.5          1.0857      7    XXXXXXX
     1.0        2.1       3.7          1.8714     39    XXXXXXXXXXXXXXXXX
     1.0        2.1       5.8R         2.6571     23    XXXXXXXXXXXXXXXXX
     1.1        2.1                    3.4428      3    XXX
     1.2        2.2                    4.2285      0
     1.3        2.2                    5.0142      0
     1.4        2.2                    5.7999      1    X
     1.5        2.2
     1.5        2.2
     1.6        2.2
     1.6        2.2
     1.6        2.3
     1.6        2.3
     1.6        2.3
     1.7        2.3
     1.8        2.3
     1.8        2.3
     1.8        2.3
     1.8        2.3
     1.8        2.4
     1.9        2.4
     1.9        2.4
     1.9        2.4
     2.0        2.5
     2.0        2.5
     2.0        2.5
     2.0        2.5
     2.0        2.6
     2.0        2.6
     2.0        2.6
     2.0        2.6
     2.0        2.7
     2.0        2.8
     2.0        3.0

R = REJECTED DATA.
```

FIGURE 7
BOD DATA, STATISTICAL SUMMARY. PREPARED BY COMPUTER FOR DIRECT USE IN REPORT.
FROM: METHOD RESEARCH STUDY 3, DEMAND ANALYSES

FIGURE 8
AMMONIA NITROGEN DATA, YOUDEN'S TWO-SAMPLE CHARTS.
PREPARED FOR REPORT BY COMPUTER PLOTTER



VOLUMETRIC ANALYSIS OF WATER QUALITY

I INTRODUCTION

A A standard solution is one whose composition and concentration are known to a high degree of accuracy. In chemical analyses, it is used to determine the concentration of a particular component in a sample.

B These chemical analyses very frequently involve an acid-base reaction or an oxidation-reduction reaction.

C Three important terms connected with these two types of chemical reactions are: mole, equivalent weight, and normality. These terms will be defined in following sections.

II ANALYTICAL CHEMICAL REACTIONS

A As stated above, in a chemical analysis a volume of standard solution is brought into contact with a volume of sample in order to determine the concentration of some particular component of the sample.

B For a given volume of sample, it is necessary to use a definite amount of the standard solution--too much or too little would give erroneous results.

C For example, one cannot simply mix together random volumes of sodium hydroxide and hydrochloric acid solutions and expect the only two substances produced to be sodium chloride and water.

D Unless the concentrations of the two reagents are known and the amounts measured accurately, excess sodium hydroxide or hydrochloric acid will also remain at the end of the reaction.

E The reason for these limitations is that when molecules react with one another, they do so in definite ratio. Unless the number of molecules of each reactant is known, there will always be an excess of one of the reactants remaining at the end of the chemical reaction. As mentioned before, this leads to erroneous analytical results.

F Because of their size, it is not possible to count out numbers of molecules. However, the number of molecules in a quantity of a chemical may be found by determining its weight and consulting a table which lists the weights of the atoms making up the chemical.

G For example, sodium hydroxide has the formula NaOH. It can also be stated that a molecule of sodium hydroxide consists of one sodium atom, one hydrogen atom and one oxygen atom.

One sodium atom weighs 23 atomic mass units (amu). An oxygen atom weighs 16 amu; and a hydrogen atom weighs 1 amu.

A molecule of sodium hydroxide, therefore, weighs 40 amu; all amu values have been rounded off. Forty amu is the molecular weight of sodium hydroxide.

H A mole of any chemical is a number of grams numerically equal to the molecular weight of that chemical. One mole of sodium hydroxide, therefore, contains 40 grams (40 g).

I Similarly, the atomic weight of a chlorine atom is 35 amu; that of a hydrogen atom is 1 amu; and the molecular weight of the hydrogen chloride molecule is 36 amu. One mole of hydrogen chloride weighs 36 g.

J Synonyms for mole are: mol, gram mol, gram mole, and gram molecular weight.

K Tables listing atomic weights of the elements can be found in virtually all of the texts used for high school and first year college chemistry courses.

L The remaining two terms mentioned in IC (equivalent weight and normality) will be considered as they are related to acid-base and oxidation-reduction reactions.

III ACID-BASE REACTIONS

A Recall that an acid is a substance which donates a hydrogen ion, or proton, (H^+) in a chemical reaction and a base is a substance which donates a hydroxide ion (OH^-) in a chemical reaction. Reactions involving these two ions are termed acid-base reactions.

B In the case of sodium hydroxide, the molecular weight is 40 amu and one mole of sodium hydroxide weighs 40 g. One hydroxide ion is contained in the sodium hydroxide molecule.

1 For a base, the number of grams in a mole divided by the number of hydroxide ions equals a quantity called the equivalent weight. Examples are given below. All amu values have been rounded off.

a Base - potassium hydroxide KOH

Atoms	Number	Wt/atom	Total
K	1	39 amu	39 amu
O	1	16	16
H	1	1	1
			56 amu

One mole of KOH = 56 g
Number of hydroxide ions = 1
Equivalent weight of KOH = 56 g

b Base - magnesium hydroxide $Mg(OH)_2$

Atoms	Number	Wt/atom	Total
Mg	1	24 amu	24 amu
O	2	16	32
H	2	1	2
			58 amu

One mole of $Mg(OH)_2$ = 58 g
Number of hydroxide ions = 2
Equivalent weight of $Mg(OH)_2$ = 29 g

2 For an acid, the number of grams in a mole divided by the number of hydrogen ions is the equivalent weight. Examples are given below. All amu values have been rounded off.

a Acid - nitric acid HNO_3

Atoms	Number	Wt/atom	Total
H	1	1 amu	1 amu
N	1	14	14
O	3	16	48
			63 amu

One mole of HNO_3 = 63 g
Number of hydrogen ions = 1
Equivalent weight of HNO_3 = 63 g

b Acid - sulfuric acid H_2SO_4

Atoms	Number	Wt/atom	Total
H	2	1 amu	2 amu
S	1	32	32
O	4	16	64
			98 amu

One mole of H_2SO_4 = 98 g
Number of hydrogen ions = 2
Equivalent weight of H_2SO_4 = 49 g

C Normality is a method of expressing solution concentrations. If one equivalent weight of a chemical is dissolved in a solvent and the volume brought to one liter (l), the concentration of the solution is one normal (N).

1 The equivalent weight of KOH was calculated to be 56 g. This amount of the solid dissolved in water and diluted to a liter would give a 1 N solution.

2 The equivalent weight of HNO_3 was found to be 63 g. This quantity of acid diluted to a liter would give a 1 N solution.

IV OXIDATION-REDUCTION REACTIONS

A The concepts of mole, equivalent weight and normality, as described in previous sections, apply also to oxidation-reduction reactions.

B One definition of an oxidation is that it involves an increase in the oxidation state (charge) of an atom.

For example: $FeCl_2 \rightarrow FeCl_3$. In this conversion the Fe has been oxidized from +2 to +3.

C A reduction is a decrease in the oxidation state (charge) of an atom.

For example: $KMnO_4 \rightarrow MnO_2$. In this conversion the Mn has been reduced from +7 to +4.

D The equivalent weight of an oxidizing or reducing agent is calculated by dividing the number of grams in a mole of the reagent by the change in charge involved.

For example: $FeCl_2$ (used as a reducing agent).

Atoms	Number	Wt/atom	Total
Fe	1	56 amu	56 amu
Cl	2	70	70
			126 amu

One mole of $FeCl_2$ = 126 g
Change in charge = 1
Equivalent weight of $FeCl_2$ = 126 g

The concentration of a liter of solution which contains 126 g of $FeCl_2$ is 1 N.

V PRIMARY STANDARDS

A A reagent of known purity is called a primary standard. Primary standard grade chemicals are available from chemical supply houses and the National Bureau of Standards. An accurately measured quantity of a primary standard is used for the preparation of standard solutions.

B Other requirements of a primary standare are:

1 It must be stable at 105° C (the temperature used for drying).

2 It should not be reactive with components of the air, such as O_2 and CO_2.

3 It should have a high equivalent weight so as to minimize any errors in the analysis.

4 It should be readily available at a reasonable cost.

VI STORAGE OF STANDARD SOLUTIONS

A Standard solutions should be prepared using high quality distilled water.

B Care should be taken to insure the cleanliness of the glass or plastic bottle used for storage.

C Some solutions may decompose on exposure to light and should be stored in dark bottles.

D The stopper or cap should fit tightly.

VII CALCULATIONS

A The basic formula used in volumetric analysis is

$$(l \times N) \text{ of standard solution} = (l \times N) \text{ of sample}$$

In a typical analysis, three of the four quantities are known, or found, and

$$N \text{ of sample} = \frac{(l \times N) \text{ of standard}}{l \text{ of sample}}$$

B This formula can be rearranged to give

$$g = l \times N \times \text{equivalent weight}$$

where g and equivalent weight (ew) refer to the component being analyzed and l and N to the standard solution.

C For example: How many g of NaOH are present in a sample if 100.0 ml of 0.2 N HCl are required for its titration?

$$g = l \times N \times \text{equivalent weight} =$$

$$\frac{100.0 \text{ ml}}{1000.0 \text{ ml}/l} \times 0.2 \times \frac{40.0}{1} = 0.8$$

REFERENCES

1 Hamilton, S.B. and Simpson, S.G. Quantitative Chemical Analysis 11th edition, The MacMillan Co., New York. 1958.

2 Blaedel, W.J. and Meloche, V.W. Elementary Quantitative Analysis: Theory and Practice. Row, Peterson and Co., N.Y. 1957.

3 Ayres, G.H. Quantitative Chemical Analysis. Harper and Brothers, New York. 1958.

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Descriptors: Analytical Techniques, Chemical Analysis, Volumetric Analysis, Water Analysis

ACIDITY, ALKALINITY, pH AND BUFFERS

I DEFINITIONS OF ACIDS AND BASES

A Arrhenius Theory of Acids and Bases (Developed about 1887)

1 Acid: A substance which produces, in aqueous solution, a hydrogen ion (proton), H^+.

2 Base: A substance which produces, in aqueous solution, a hydroxide ion, OH^-.

3 The Arrhenius theory was confined to the use of water as a solvent.

B Bronsted and Lowry Theory of Acids and Bases (Developed about 1923)

1 Acid: A substance which donates, in chemical reaction, a hydrogen ion (proton).

2 Base: A substance which accepts, in chemical reaction, a hydrogen ion (proton).

3 Bronsted and Lowry had expanded the acid-base concept into non-aqueous media; i.e., the solvent could, but did not have to be, water.

C There are other acid-base theories. The two above are probably the most commonly used ones when discussing wastewater topics however.

II DEFINITIONS OF ACIDITY, ALKALINITY AND NEUTRALITY

A Acidity

A condition in which there is a preponderance of acid materials present in the water.

B Alkalinity

A condition in which there is a preponderance of alkaline (or basic) materials present in the water.

C Neutrality

1 It is possible to have present in the water chemically equivalent amounts of acids and bases. The water would then be described as being neutral; i.e., there is a preponderance of neither acid nor basic materials. The occurrence of such a condition would be rare.

2 The term "neutralization" refers to the combining of chemically equivalent amounts of acids and bases. The two products of neutralization are a salt and water.

$$HCl + NaOH = NaCl + H_2O$$

Hydrochloric acid	Sodium Hydroxide	Sodium Chloride (a salt)

D The key word in the above definitions is "preponderance." It is possible to have a bas ion of acidity while there are basic materials present in the water, as well as conversely.

III HOW ARE DEGREES OF ACIDITY AND ALKALINITY EXPRESSED?

The pH scale is used to express various degrees of acidity and alkalinity. Values can range from 0 to 14. These two extremes are of theoretical interest and would never be encountered in a natural water or in a waste water. pH readings from 0 to just under 7 indicate an acidic condition; from just over 7 to 14, an alkaline condition. Neutrality exists if the pH value is exactly 7. pH paper, or a pH meter, provides the most convenient method of obtaining pH readings. It should be noted, that under NPDES Methodology, pH measurements are to be made using a pH meter. Some common liquids and pH values are listed in Table I.

TABLE 1. pH Values of Common Liquids

Household lye	13.7
Bleach	12.7
Ammonia	11.3
Milk of magnesia	10.2
Borax	9.2
Baking soda	8.3
Sea water	8.0
Blood	7.3
Distilled water	7.0
Milk	6.8
Corn	6.2
Boric acid	5.0
Orange juice	4.2
Vinegar	2.8
Lemon juice	2.2
Battery acid	0.2

5.9 - 8.4 is the common pH range for most natural waters.

IV HARD AND SOFT WATERS

In addition to being acidic or basic, water can also be described as being hard or soft.

A Hard water contains large amounts of calcium, magnesium, strontium, manganese and iron ions, relative to the amount of sodium and potassium ions present. Hard water is objectionable because it forms insoluble compounds with ordinary soap.

B Soft water contains small amounts of calcium, magnesium, strontium, manganese and iron ions, relative to the amount of sodium and potassium ions present. Soft water does not form insoluble compounds with ordinary soap.

V TITRATIONS

A The conversion of pH readings into such quantities as milligrams (mg) of acidity, alkalinity, or hardness, is not easily carried out. These values are more easily obtained by means of a titration.

B In a titration, an accurately measured volume of sample (of unknown strength) is combined with an accurately measured volume of standard solution (of known strength) in the presence of a suitable indicator.

C The strength (called normality) of the sample is then found using the following expression:

milliliters (ml) of sample × normality (N) of sample = ml of standard solution × N of standard solution.

Three of the four quantities are known, and

N of sample = ml of standard solution × N of standard solution/ml of sample.

D In modified form, and a more specific application of the above equation, alkalinity is calculated in the following manner (13th ed. Standard Methods).

mg of alkalinity as mg $CaCO_3$/liter (l) = ml of standard H_2SO_4 × N of standard H_2SO_4 × 50 × 1000/ml sample.

VI INDICATORS

The term "suitable indicator" was used above. At the end of a titration, the pH of the solution will not necessarily be 7. It may be above or below 7. A suitable indicator, therefore, is one which undergoes its characteristic color change at the appropriate pH. Below are a few examples of indicators and the pH range in which they undergo their characteristic color changes. In some cases, mixed indicators may be used in order to obtain a sharper and more definite color change. Again it should be noted that under NPDES, pH meters are to be used for the measurement of pH.

Indicator	Operational pH Range
Methyl Yellow	2.8 - 4.0
Methyl Orange	3.1 - 4.4
Methyl Red	4.4 - 6.2
Cresol Purple	7.4 - 9.0
Phenolphthalein	8.0 - 9.6
Alizarine Yellow	10.0 - 12.0

TABLE 2. pH Range of Indicators

VII BUFFERS

A A buffer is a combination of substances which, when dissolved in water, resists a pH change in the water, as might be caused by the addition of acid or alkali. Listed below are a few chemicals which, when combined in the proper proportions, will tend to maintain the pH in the indicated range.

Chemicals	pH Range
Acetic Acid + Sodium Acetate	3.7 - 5.6
Sodium Dihydrogen Phosphate + Disodium Hydrogen Phosphate	5.8 - 8.0
Boric Acid + Borax	6.8 - 9.2
Borax + Sodium Hydroxide	9.2 - 11.0

TABLE 3. pH Range of Buffers

B A buffer functions by supplying ions which will react with hydrogen ions (acid "spill"), or with hydroxide ions (alkali "spill").

C In many instances, the buffer is composed of a weak acid and a salt of the weak acid; e.g., acetic acid and sodium acetate.

1 In water, acetic acid ionizes or "breaks down" into hydrogen ions and acetate ions.

$HC_2H_3O_2$	=	H^+	+	$C_2H_3O_2^-$
(acetic acid)		(hydrogen ion) (proton)		(acetate ion)

This ionization occurs to only a slight extent, however, most of the acetic acid remains in the form of $HC_2H_3O_2$; only a small amount of hydrogen and acetate ions is formed.

2 Thus, acetic acid is said to be a weak acid.

3 In the case of other acids, ionization into the component ions occurs to a large degree, and the term strong acid is applied; e.g., hydrochloric acid.

HCl	=	H^+	+	Cl^-
(hydrochloric acid)		(hydrogen ion) (proton)		(chloride ion)

4 The terms "strong" and "weak" are also applied to bases. In water solutions, those which break down into their component ions to a large extent are termed "strong", and those which do not are "weak". Sodium hydroxide is a relatively strong base, while ammonium hydroxide is realtively weak.

5 Sodium acetate (a salt of acetic acid) dissociates or "breaks down" into sodium ions and acetate ions when placed in water.

$NaC_2H_3O_2$	=	Na^+	+	$C_2H_3O_2^-$
(sodium acetate)		(sodium ion)		(acetate ion)

This dissociation occurs to a large extent, and practically all of the sodium acetate is in the form of sodium ions and acetate ions.

D It would be difficult and expensive to prepare large quantities of buffers for use in a treatment plant. However, certain naturally occurring buffers may be available (carbon dioxide is an example). It dissolves in water to form the species indicated below.

CO_2	+	H_2O	=	H_2CO_3
(carbon dioxide)		(Water)		(carbonic acid)

H_2CO_3	=	H^+	+	HCO_3^-
		(hydrogen ion) (proton)		(hydrogen carbonate ion) (bicarbonate)

The hydrogen ions react with hydroxide ions which might appear in the water as the result of an alkali "spill".

H^+	+	OH^-	=	H_2O
(in the buffer)		(hydroxide ion "spilled")		

The hydrogen carbonate ions react with hydrogen ions which might appear in the water as the result of an acid "spill".

H^+	+	HCO_3^-	=	H_2CO_3
(hydrogen ion) (proton) "spilled"		(in the buffer)		

This buffering action will be in effect as long as there is carbonic acid present.

E Buffering action is not identical with a process in which acid wastes are "neutralized" with alkali wastes, or conversely. The desired effect is achieved in both cases, however (i.e., the pH is maintained within a desired range.)

This outline was prepared by C. R. Feldmann, Chemist, National Training Center, MPOD, OWPO, EPA, Cincinnati, OH 45268

Descriptors: Acids, Acidity, Alkalis, Alkalinity, Analytical Techniques, Buffers, Buffering Capacity, Chemical Analysis, Hydrogen Ion Concentration, Indicators, Neutralization, Water Analysis.

ALKALINITY AND RELATIONSHIPS AMONG THE VARIOUS TYPES OF ALKALINITIES

I PRELIMINARY

The property of water referred to as alkalinity is usually caused by the presence of hydroxyl, carbonate and bicarbonate ions. To a lesser extent, borates, phosphates and silicates contribute but are generally present in negligible amounts.

The concentration and ratio of the OH^-, CO_3^{--}, and HCO_3^- ions may be measured by titrating a sample to certain specified pH's or end points which are detected either by use of a pH meter or by color indicators. Phenolphthalein is used for visual detection of the first end point, (approximately pH 8) which indicates the neutralization of NaOH and conversion of CO_3^{--} to HCO_3^-. A number of indicators (methyl orange, methyl purple, brom cresol green, etc.) are used for detection of the second end point (pH 3-5) which indicates the complete conversion of HCO_3^- to H_2O and CO_2. The final end point is determined by the amount of CO_3^{--} and HCO_3^- originally present in the sample. If the end points are determined electrometrically they are taken as the mid-point of the greatest rate of pH change per unit volume of titrant.

II RELATIONSHIPS BETWEEN HYDROXIDE, CARBONATE, AND BICARBONATE ALKALINITIES

The results obtained from phenolphthalein and total alkalinity measurements offer a means of classification of the principal forms of alkalinity, if certain assumptions are made. It must first be assumed that interferences are absent and that bicarbonate and hydroxide do not exist in the same solution. According to the system presented in Standard Methods, 13th Edition:

A Hydroxide alkalinity is present if the phenolphthalein alkalinity is more than one-half the total alkalinity.

B Carbonate alkalinity is present if the phenolphthalein alkalinity is not zero but is less than the total alkalinity.

C Bicarbonate alkalinity is present if the phenolphthalein alkalinity is less than one-half the total alkalinity.

Table 1. Relationships Between Phenolphthalein Alkalinity, Total Alkalinity, Carbonate Alkalinity, Bicarbonate Alkalinity and Hydroxide Alkalinity

Lecture Notes	Result of Titration	OH^- Alkalinity as $CaCO_3$	CO_3^{--} Alkalinity as $CaCO_3$	HCO_3^- Alkalinity as $CaCO_3$
Case 1	P = T	T	0	0
Case 2	P = ½T	0	2P	0
Case 3	P = 0	0	0	T
Case 4	P > ½T	2P-T	2(T-P)	0
Case 5	P < ½T	0	2P	T-2P

P = Phenolphthalein Alkalinity T = Total Alkalinity

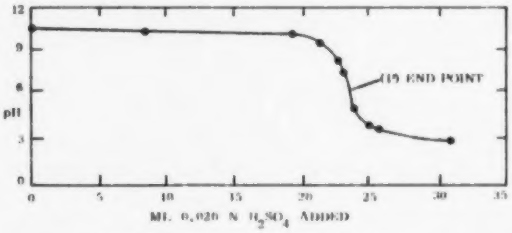
Table 2. Stoichiometric Volumes of Solutions of Different Normalities

Standard Solution	H_2SO_4	NaOH	Na_2CO_3	$NaHCO_3$
Normality	0.0200	0.0189	0.0199	0.0125
Equivalent Volumes, ml	10.0	10.6	9.9	16.0
	9.4	10.0	9.5	15.1
	10.0	10.5	10.0	15.9
	6.3	6.6	6.3	10.0

III CASE EXAMPLES

The relationships involved in Table 1 may best be explained by reference to the following graphs. These were prepared by titrating volumes of standard solutions of sodium hydroxide, sodium carbonate, and sodium bicarbonate with standard sulfuric acid. The stoichiometric volumes of the various solutions are summarized in Table 2 for convenience in the interpretation of the charts.

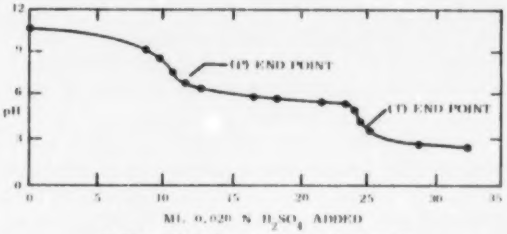
A CASE 1 - Where phenolphthalein alkalinity = total alkalinity



25 ML. 0.0189 N NaOH vs 0.020 N H_2SO_4
(P = T)

The sharp break occurs at the point where all of the NaOH has been exactly neutralized by the acid. The pH and concentration of the end products (Na_2SO_4 and H_2O) determine the pH at the equivalence point between NaOH and H_2SO_4; in this case, approximately 7.0.

B CASE 2 - Where phenolphthalein alkalinity = one-half the total alkalinity

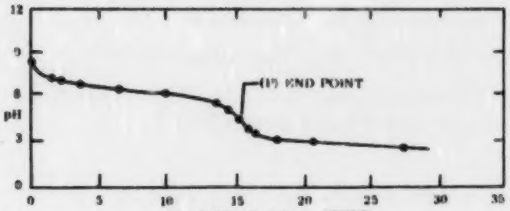


25 ML. 0.020 N Na_2CO_3 vs 0.020 N H_2SO_4
(P = ½ T)

The titration proceeds in 2 stages wherein all of the CO_3^{--} is converted, first to HCO_3^- and finally to H_2CO_3. The first end point occurs at approximately pH 8, and at exactly half the volume of acid used for the total titration. The end point which occurs at approximately pH 4 represents the total alkalinity and requires exactly twice the volume of acid used for the first end point.

If either HCO_3^- or OH^- ions had been present the titration volumes for the curves would not have been of equal magnitude.

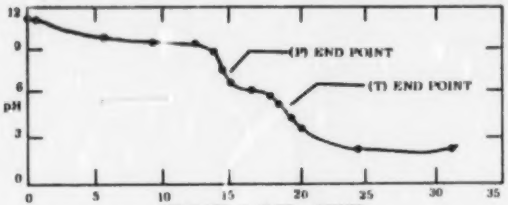
C CASE 3 - Where phenolphthalein alkalinity = 0



25 ML 0.0125 N $NaHCO_3$ vs 0.020 N H_2SO_4
(P = 0)

T The reaction proceeds in one stage with the initial pH at approximately 8.5 and final pH at 4.0. In this case the phenolphthalein alkalinity is zero and since no conversion of CO_3^{--} to HCO_3^- is noted the total alkalinity can only be due to the HCO_3^- ion.

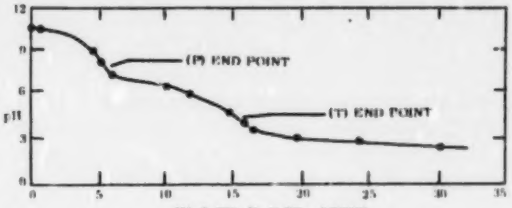
D CASE 4 - Where phenolphthalein alkalinity is greater than one-half the total alkalinity



[10 ML 0.0180 N NaOH + 10 ML 0.020 N Na_2CO_3] vs 0.020 N H_2SO_4
(P > ½ T)

The volume of acid required for the first end point (phenolphthalein alkalinity) is due to the OH^- neutralization and conversion of the CO_3^{--} to HCO_3^-. The second end point represents the complete conversion of HCO_3^- to H_2CO_3. Referring to Case 2 where the volume of acid was similar for each end point, it is apparent that a base responding to phenolphthalein but which is not CO_3^{--} must be present. Since it was originally assumed that OH^- and HCO_3^- do not exist in the same solution we must conclude that the total alkalinity is due to OH^- and CO_3^{--}.

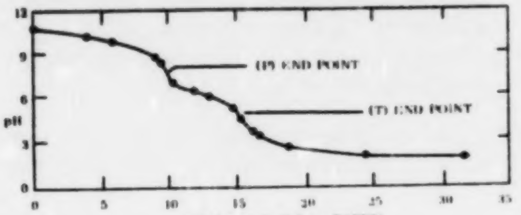
E CASE 5 - Where phenolphthalein alkalinity is less than one half of the total alkalinity



[10 ML 0.020 N Na_2CO_3 + 10 ML 0.125 N $NaHCO_3$] vs 0.020 N H_2SO_4
(P < ½ T)

If, in the reaction $NaOH + NaHCO_3 \rightarrow Na_2CO_3 + H_2O$, the NaOH exists in excess quantity, the final sample contains NaOH and Na_2CO_3, (Case 4) in which the volume of acid required for the phenolphthalein end point is greater than one half the total. In this case, however, the situation is reversed, wherein the volume of acid required for the HCO_3^- end point is greater than one half the total. Referring again to the reaction $NaOH + NaHCO_3 \rightarrow Na_2CO_3 + H_2O$, if $NaHCO_3$ is in excess the end products must consist of Na_2CO_3 and $NaHCO_3$ and OH^- must be absent. The end points consist, therefore, of $Na_2CO_3 \rightarrow NaHCO_3$ (phenolphthalein end point) and $NaHCO_3 \rightarrow H_2CO_3$.

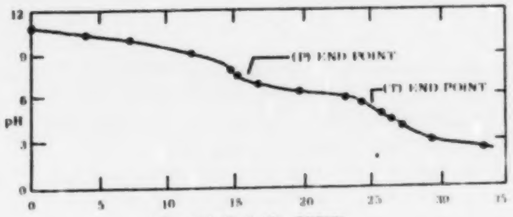
F CASE 6 - Where phenolphthalein alkalinity is greater than one-half total alkalinity.



[10 ML 0.0189 N NaOH + 10 ML 0.0125 N $NaHCO_3$] vs 0.020 N H_2SO_4
($P > \frac{1}{2}T$)

Following the original assumption that OH^- and HCO_3^- are not compatible, with HCO_3^- being converted to CO_3^{--} we have a condition similar to Case 4 ($P > \frac{1}{2}T$).

G CASE 7 - Where phenolphthalein alkalinity is greater than one-half total alkalinity.



[10ML 0.0189 N NaOH + 10 ML 0.020 N Na_2CO_3 + 10 ML 0.0125 N $NaHCO_3$] vs 0.020 N H_2SO_4
($P > \frac{1}{2}T$)

The first end point occurs at the stoichiometric sum of the equivalent volumes as follows:

$$(9.4 - 6.3) + \frac{(10 + 12.6)}{2}$$

where (9.4 - 6.3) = volume of N acid required for excess OH^- after OH^- + HCO_3^- reaction,

and $\frac{(10 + 12.6)}{2}$ = volume of acid required for conversion of CO_3^{--} to HCO_3^-. The second end point occurs at $\frac{(10 + 12.6)}{2}$ ml, the volume of HCO_3^- which is converted to H_2CO_3. This then becomes the same as Case 4.

IV COMPARISON OF ANALYTICAL METHODS FOR ALKALINITY (According to the Analytical Reference Service Report JAWWA Vol. 55, No. 5, 1963)

May 1963 DETERMINATION METHODS

TABLE 2--*Statistical Summary (contd.)*

Method	Year	No. of Values Reported	Concn. Added mg/l	Concn. Determined mg/l			99% Range mg/l	Standard Deviation mg/l
				Mean	Low	High		
Alkalinity								
Methyl orange	1956	38	19	20.0	11.23	30.0	± 2	5.049
	1958	19	17	19.5	16	24	± 1	2.483
	1961	27	42.5	43.7	38	50	± 1.5	3.250
Electrometric	1956	14	19	19.7	19.0	21.0	± 1	0.911
	1958	53	17	19.4	15	25	± 2	2.740
	1961	88	42.5	44.2	38.9	57	± 2.5	3.605
Methyl purple	1956	8	19	14.7	14.0	15.2	± 4	0.475
	1958	10	17	19.3	18	21	± 2	0.823
	1961	18	42.5	44.8	40	50	± 1.5	3.081
Mixed indicator	1958	4	17	19.5	18	20	± 3	1.000
	1961	15	42.5	42.8	39	49	± 3	3.550
Brom cresol green	1958	1	17	19	19	19	± 2	
All methods	1956	60	19	19.2	11.23	30.0	± 2	4.460
	1958	92	17	19.6	15	25	± 2	2.320
	1961	178	42.5	43.9	25	57	± 1.9	5.335

Alkalinity - The methods for alkalinity measurement varied only in the choice of indicator or pH for determining the end point of the titration. The indicators used included methyl orange, methyl purple, and mixed indicator. The data shows that as the use of electrometric end point increased, the use of methyl orange decreased.

SUMMARY	
Amount Added	17 mg/l (as $CaCO_3$)
Avg. Deviation from amount added	3.1 mg/l
Standard Deviation	3.4 mg/l
50% Range	2.0 mg/l
Method Most Commonly Used	Electrometric
Method Preferred	Electrometric
Total Number of Observations	41

V PROCEDURE

The actual measurement of alkalinity is a very simple procedure requiring only titration of sample with a standardized acid and the proper indicator. For phenolphthalein alkalinity the end point and indicator are well established. For the bicarbonate titration the final end point is a function of the HCO_3^- concentration. With low amounts (< 50 mg/l as $CaCO_3$) the pH at end point may be approximately 5.0. With high concentrations (> 250 mg/l as $CaCO_3$) the pH at end point may be 4.5 to 3.8. For all-purpose work, in which the highest degree of accuracy is not required, an end point at pH 4.5 using methyl purple as the indicator is recommended.

The traditional methyl orange frequently proves to be unsatisfactory because of the indefinite color change at the end point and also because of the low pH (3.8 - 3.9) required to establish the change.

VI EPA ANALYTICAL METHODS.

A The Environmental Protection Agency, Office of Water Programs, Analytical Quality Control Laboratory has compiled a manual of Analytical Methods which is to be used in Federal laboratories for the chemical analysis of water and waste samples. The title of this manual is "Methods for Chemical Analysis of Water and Wastes, 1971."

B This manual lists two parameters which are related to the subject matter in this outline. They are total alkalinity and total acidity.

C For the measurement of both of these parameters, the recommended method is volumetric, with the equivalence point being determined electrometrically. The use of a color indicator (methyl orange) is recommended only for the automated method.

D The procedure references cited by the EPA Methods manual are the 13th Edition of Standards Methods and ASTM Standards, Part 23, 1970.

REFERENCES

1 Standards Methods for the Examination of Water and Wastewater, 13th Ed. APHA, Inc., New York. 1971.

2 ASTM Standards, Part 23. 1973.

This outline was prepared by R. C. Kroner, former Chief, Physical and Chemical Methods, Analytical Quality Control Laboratory, National Environmental Research Center, Cincinnati, OH 45268.

Descriptors: Alkalis, Alkalinity, Analytical Techniques, Buffers, Buffering Capacity, Chemical Analysis, Water Analysis

DISSOLVED OXYGEN

Factors Affecting DO Concentration in Water

I The Dissolved Oxygen determination is a very important water quality criteria for many reasons:

A Oxygen is an essential nutrient for all living organisms. Dissolved oxygen is essential for survival of aerobic organisms and permits facultative organisms to metabolize more effectively. Many desirable varieties of macro or micro organisms cannot survive at dissolved oxygen concentrations below certain minimum values. These values vary with the type of organisms, stage in their life history, activity, and other factors.

B Dissolved oxygen levels may be used as an indicator of pollution by oxygen demanding wastes. Low DO concentrations are likely to be associated with low quality waters.

C The presence of dissolved oxygen prevents or minimizes the onset of putrefactive decomposition and the production of objectionable amounts of malodorous sulfides, mercaptans, amines, etc.

D Dissolved oxygen is essential for terminal stabilization wastewaters. High DO concentrations are normally associated with good quality water.

E Dissolved oxygen changes with respect to time, depth or section of a water mass are useful to indicate the degree of stability or mixing characteristics of that situation.

F The BOD or other respirometric test methods for water quality are commonly based upon the difference between an initial and final DO determination for a given sample time interval and condition. These measurements are useful to indicate:

1 The rate of biochemical activity in terms of oxygen demand for a given sample and conditions.

2 The degree of acceptability (a bioassay technique) for biochemical stabilization of a given microbiota in response to food, inhibitory agents or test conditions.

3 The degree of instability of a water mass on the basis of test sample DO changes over an extended interval of time.

4 Permissible load variations in surface water or treatment units in terms of DO depletion versus time, concentration, or ratio of food to organism mass, solids, or volume ratios.

5 Oxygenation requirements necessary to meet the oxygen demand in treatment units or surface water situations.

G The DO test is the only chemical test included in all Water Quality Criteria, Federal, State, Regional or local.

II FACTORS AFFECTING THE DO CONCENTRATION IN WATER

A Physical Factors:

1 DO solubility in water for an air/water system is limited to about 9 mg DO/liter of water at 20°C. This amounts to about 0.0009% as compared to 21% by weight of oxygen in air.

2 Transfer of oxygen from air to water is limited by the interface area, the oxygen deficit, partial pressure, the conditions at the

interface area, mixing phenomena and other items.

Certain factors tend to confuse reoxygenation mechanisms of water aeration:

a The transfer of oxygen in air to dissolved molecular oxygen in water has two principal variables:

1) Area of the air-water interface.

2) Dispersion of the oxygen-saturated water at the interface into the body liquid.

The first depends upon the surface area of the air bubbles in the water or water drops in the air; the second depends upon the mixing energy in the liquid. If diffusors are placed in a line along the wall, dead spots may develop in the core. Different diffusor placement or mixing energy may improve oxygen transfer to the liquid two or threefold.

b Other variables in oxygen transfer include:

3) Oxygen deficit in the liquid.

4) Oxygen content of the gas phase.

5) Time.

If the first four variables are favorable, the process of water oxygenation is rapid until the liquid approaches saturation. Much more energy and time are required to increase oxygen saturation from about 95 to 100% than to increase oxygen saturation from 0 to about 95%. For example: An oxygen-depleted sample often will pick up significant DO during DO testing; changes are unlikely with a sample containing equilibrium amounts of DO.

c The limited solubility of oxygen in water compared to the oxygen content of air does not require the interchange of a large mass of oxygen per unit volume of water to change DO saturation. DO increases from zero to 50% saturation are common in passage over a weir.

d Aeration of dirty water is practiced for cleanup. Aeration of clean water results in washing the air and transferring fine particulates and gaseous contaminants to the liquid.

e One liter of air at room temperature contains about 230 mg of oxygen. A 5 gal carboy of water with 2 liters of gas space above the liquid has ample oxygen supply for equilibration of DO after storage for 2 or 3 days or shaking for 30 sec.

f Aeration tends toward evaporative cooling. Oxygen content becomes higher than saturation values at the test temperature, thus contributing to high blanks.

3 Oxygen solubility varies with the temperature of the water. Solubility at 10° C is about two times that at 30° C. Temperature often contributes to DO variations much greater than anticipated by

solubility. A cold water often has much more DO than the solubility limits at laboratory temperature. Standing during warmup commonly results in a loss of DO due to oxygen diffusion from the supersaturated sample. Samples warmer than laboratory temperature may decrease in volume due to the contraction of liquid as temperature is lowered. The full bottle at higher temperature will be partially full after shrinkage with air entrance around the stopper to replace the void. Oxygen in the air may be transferred to raise the sample DO. For example, a volumetric flask filled to the 1000 ml mark at 30° C will show a water level about 1/2 inch below the mark when the water temperature is reduced to 20° C. BOD dilutions should be adjusted to 20° C + or - 1 1/2° before filling and testing.

4 Water density varies with temperature with maximum water density at 4° C. Colder or warmer waters tend to promote stratification of water that interferes with distribution of DO because the higher density waters tend to seek the lower levels.

5 Oxygen diffusion in a water mass is relatively slow, hence vertical and lateral mixing are essential to maintain relatively uniform oxygen concentrations in a water mass.

6 Increasing salt concentration decreases oxygen solubility slightly but has a larger effect upon density stratification in a water mass.

7 The partial pressure of the oxygen in the gas above the water interface controls the oxygen solubility limits in the water. For example, the equilibrium concentration of oxygen in water is about 9 mg DO/l under one atmospheric pressure of air, about 42 mg DO/liter in contact with pure oxygen and 0 mg DO/liter in contact with pure nitrogen (@ 20° C).

B Biological or Bio-Chemical Factors

1 Aquatic life requires oxygen for respiration to meet energy requirements for growth, reproduction, and motion. The net effect is to deplete oxygen resources in the water at a rate controlled by the type, activity, and mass of living materials present, the availability of food and favorability of conditions.

2 Algae, autotrophic bacteria, plants or other organisms capable of photosynthesis may use light energy to synthesize cell materials from mineralized nutrients with oxygen released in process.

a Photosynthesis occurs only under the influence of adequate light intensity.

b Respiration of alga is continuous.

c The dominant effect in terms of oxygen assets or liabilities of alga depends upon algal activity, numbers and light intensity. Gross algal productivity contributes to significant diurnal DO variations.

3 High rate deoxygenation commonly accompanies assimilation of readily available nutrients and conversion into cell mass or storage products. Deoxygenation due to cell mass respiration commonly occurs at some lower rate dependent upon the nature of the organisms present, the stage of decomposition and the degree of predation, lysis, mixing and regrowth. Relatively high

deoxygenation rates commonly are associated with significant growth or regrowth of organisms.

4 Micro-organisms tend to flocculate or agglomerate to form settleable masses particularly at limiting nutrient levels (after available nutrients have been assimilated or the number of organisms are large in proportion to available food).

a Resulting benthic deposits continue to respire as bed loads.

b Oxygen availability is limited because the deposit is physically removed from the source of surface oxygenation and algal activity usually is more favorable near the surface. Stratification is likely to limit oxygen transfer to the bed load vicinity.

c The bed load commonly is oxygen deficient and decomposes by anaerobic action.

d Anaerobic action commonly is characterized by a dominant hydrolytic or solubilizing action with relatively low rate growth of organisms.

e The net effect is to produce low molecular weight products from cell mass with a correspondingly large fraction of feedback of nutrients to the overlaying waters. These lysis products have the effect of a high rate or immediate oxygen demand upon mixture with oxygen containing waters.

f Turbulence favoring mixing of surface waters and benthic sediments commonly are associated with extremely rapid depletion of DO. Recurrent resuspension of thin benthic deposits may contribute to highly erratic DO patterns.

g Long term deposition areas commonly act like point sources of new pollution as a result of the feedback of nutrients from the deposit. Rate of reaction may be low for old materials but a low percentage of a large mass of unstable material may produce excessive oxygen demands.

C Tremendous DO variations are likely in a polluted water in reference to depth, cross section or time of day. More stabilized waters tend to show decreased DO variations although it is likely that natural deposits such as leaf mold will produce differences related to depth in stratified deep waters.

ACKNOWLEDGMENTS

This outline contains significant materials from previous outlines by J. W. Mandia. Review and comments by C. R. Hirth and R. L. Booth are greatly appreciated.

REFERENCE

1 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL. 1974.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, MPOD, OWPO, EPA, Cincinnati, OH 45268 and revised by Charles R. Feldmann, Chemist, National Training Center.

Descriptors: Aeration, Aerobic Conditions, Air-Water Interfaces, Anaerobic Conditions, Benthos, Biological Oxygen Demand, Dissolved Oxygen, Water Pollution, Water Quality

DISSOLVED OXYGEN

Determination By The Winkler Iodometric Titration and Azide Modification

The basic Winkler procedure (1888) has been modified many times to improve its workability in polluted waters. None of these modifications have been completely successful. The most useful modification was proposed by Alsterberg and consists of the addition of sodium azide to control nitrite interference during the iodometric titration. The Azide modification of the iodometric titration is recommended as official by the EPA-WPO Quality Control Committee for relatively clean waters.

A Reactions

1 The determination of DO involves a complex series of interactions that must be quantitative to provide a valid DO result. The number of sequential reactions also complicates interference control. The reactions will be presented first followed by discussion of the functional aspects.

$$\mathrm{MnSO_4 + 2\ KOH \rightarrow Mn(OH)_2 + K_2SO_4} \tag{a}$$

$$\mathrm{2\ Mn(OH)_2 + O_2 \rightarrow 2\ MnO(OH)_2} \tag{b}$$

$$\mathrm{MnO(OH)_2 + 2\ H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O} \tag{c}$$

$$\mathrm{Mn(SO_4)_2 + 2\ KI \rightarrow MnSO_4 + K_2SO_4 + I_2} \tag{d}$$

$$\mathrm{I_2 + 2\ Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2\ NaI} \tag{e}$$

2 Reaction sequence

The series of reactions involves five different operational steps in converting dissolved oxygen in the water into a form in which it can be measured.

a $\mathrm{O_2 \rightarrow MnO(OH)_2 \rightarrow Mn(SO_4)_2 \rightarrow I_2 \rightarrow}$ Thiosulfate (thio) or phenylarsine oxide (PAO) titration.

b All added reagents are in excess to improve contact possibilities and to force the reaction toward completion.

3 The first conversion, $\mathrm{O_2 \rightarrow MnO(OH)_2}$ (reactions a, b) is an oxygen transfer operation where the dissolved oxygen in the water combines with manganous hydroxide to form an oxygenated manganic hydroxide.

a The manganous salt can react with oxygen only in a highly alkaline media.

b The manganous salt and alkali must be added separately with addition below the surface of the sample to minimize reaction with atmospheric oxygen via air bubbles or surface contact. Reaction with sample dissolved oxygen is intended to occur upon mixing of the reagents and sample after stoppering the full bottle (care should be used to allow entrained air bubbles to rise to the surface before adding reagents to prevent high results due to including entrained oxygen).

c Transfer of oxygen from the dissolved state to the precipitate form involves a two phase system of solution and precipitate requiring effective mixing for quantitative transfer. Normally a gross excess of reagents is used to limit mixing requirements. Mixing by rapid inversion 25 to 35 times will accomplish the purpose. Less energy is required by inversion 5 or 6 times, allowing the solids to settle half way and repeat the process. Reaction is rapid; contact is the principal problem in the two phase system.

d If the alkaline floc is white, no oxygen is present.

4 Acidification (reactions c and d) changes the oxygenated manganic hydroxide to manganic sulfate which in turn reacts with potassium iodide to form elemental iodine. Under acid conditions, oxygen cannot react directly with the excess manganous sulfate remaining in solution.

5 Iodine (reaction e) may be titrated with sodium thiosulfate standard solution to indicate the amount of dissolved oxygen originally present in the sample.

a The blue color of the starch-iodine complex commonly is used as an indicator. This blue color disappears when elemental iodine has been reacted with an equivalent amount of thiosulfate.

b Phenylarsine oxide solutions are more expensive to obtain but have better keeping qualities than thiosulfate solutions. Occasional use, field operations and situations where it is not feasible to calibrate thio solutions regularly, usually encourage use of purchased PAO reagents.

6 For practical purposes the DO determination scheme involves the following operations.

a Fill a 300 ml bottle* under conditions minimizing DO changes. This means that the sample bottle must be flushed with test solution to displace the air in the bottle with water characteristic of the tested sample*.

*DO test bottle volumes should be checked - discard those outside of the limits of 300 ml + or - 3 ml.

b To the filled bottle:

1) Add $MnSO_4$ reagent (2 ml)

2) Add KOH, KI, NaN_3 reagent (2 ml)
Stopper, mix by inversion, allow to settle half way and repeat the operation. Highly saline test waters commonly settle very slowly at this stage and may not settle to the half way point in the time allotted.

c To the alkaline mix (settled about half way) add 2 ml of sulfuric acid, stopper and mix until the precipitate dissolves.

d Transfer the contents of the bottle to a 500 ml Erlenmeyer flask and titrate with 0.0375 Normal Thiosulfate*. Each ml of reagent used represents 1 mg of DO/liter of sample.

The same thing applies for other sample volumes when using an appropriate titrant normality such as:

1) For a 200 ml sample, use 0.025 N Thio

2) For a 100 ml sample, use 0.0125 N Thio

*EPA-WPO Method

7 The addition of the first two DO reagents, ($MnSO_4$ and the KOH, KI and NaN_3 solutions) displaces an equal quantity of the sample. This is not the case when acid is added because the clear liquid above the floc does not contain dissolved oxygen as all of it should be converted to the particulate $MnO(OH)_2$. Some error is introduced by this displacement of sample during dosage of the first two reagents. The error upon addition of 2 ml of each reagent to a 300 ml sample is $\frac{4}{300}$ × 100 or 1.33% loss in DO. This may be corrected by an appropriate factor or by adjustment of reagent normality. It is generally considered small in relation to other errors in sampling, manipulation and interference, hence this error may be recognized but not corrected.

8 Reagent preparation and procedural details can be found in reference 1.

IV The sequential reactions for the Chemical DO determination provides several situations where significant interference may occur in application on polluted water, such as:

A Sampling errors may not be strictly designated as interference but have the same effect of changing sample DO. Inadequate flushing of the bottle contents or exposure to air may raise the DO of low oxygen samples or lower the DO of supersaturated samples.

B Entrained air may be trapped in a DO bottle by:

1 Rapid filling of vigorously mixed samples without allowing the entrained air to escape before closing the bottle and adding DO reagents.

2 Filling a bottle with low temperature water holding more DO than that in equilibrium after the samples warm to working temperature.

3 Aeration is likely to cool the sample permitting more DO to be introduced than can be held at the room or incubator temperatures.

4 Samples warmer than working incubator temperatures will be only partially full at equilibrium temperatures.

Addition of DO reagents results in reaction with dissolved or entrained oxygen. Results for DO are invalid if there is any evidence of gas bubbles in the sample bottle.

C The DO reagents respond to any oxidant or reductant in the sample capable of reacting within the time allotted. HOCl or H_2O_2 may raise the DO titration while H_2S, & SH^- may react with sample oxygen to lower the sample titration. The items mentioned react rapidly and raise or lower the DO result promptly. Other items such as Fe^{++} or $SO_3^=$ may or may not react completely within the time allotted for reaction. Many organic materials or complexes from benthic deposits may have an effect upon DO results that are difficult to predict. They may have one effect during the alkaline stage to release iodine from KI while favoring irreversible absorption of iodine during the acid stage. Degree of effect may increase with reaction time. It is generally inadvisable to use the iodometric titration on samples containing large amounts of organic contaminants or benthic residues. It would be expected that benthic residues would tend toward low results because of the reduced iron and sulfur content - they commonly favor high results due to other factors that react more rapidly, often giving the same effect as in uncontrolled nitrite interference during titration.

D Nitrite is present to some extent in natural waters or partially oxidized treatment plant samples. Nitrite is associated with a cyclic reaction during the acid stage of the DO determination that may lead to erroneous high results.

1 These reactions may be represented as follows:

$$\begin{array}{ll} 2HNO_2 + 2\,HI \rightarrow I_2 + 4H_2O + N_2O_2 & (a) \\ \uparrow \qquad\qquad\qquad\qquad\qquad\qquad \downarrow & \\ 2HNO_2 \leftarrow + 1/2O_2 + H_2O + N_2O_2 & (b) \end{array}$$

These reactions are time, mixing and concentration dependent and can be minimized by rapid processing.

2 Sodium azide (NaN_3) reacts with nitrite under acid conditions to form a combination of $N_2 + N_2O$ which effectively blocks the cyclic reaction by converting the HNO_2 to noninterfering compounds of nitrogen.

3 Sodium azide added to fresh alkaline KI reagent is adequate to control interference up to about 20 mg of NO_2^- N/liter of sample. The azide is unstable and gradually decomposes. If resuspended benthic sediments are not detectable in a sample showing a returning blue color, it is likely that the azide has decomposed in the alkaline KI azide reagent.

E Surfactants, color and Fe+++ may confuse endpoint detection if present in significant quantities.

F Polluted water commonly contains significant interferences such as C. It is advisable to use a membrane protected sensor of the electronic type for DO determinations in the presence of these types of interference.

G The order of reagent addition and prompt completion of the DO determination is critical. Stable waters may give valid DO results after extended delay of titration during the acidified stage. For unstable water, undue delay at any stage of processing accentuates interference problems.

REFERENCE

1 Methods for Chemical Analysis of Water and Wastes. EPA-MDQARL. 1974.

ACKNOWLEDGMENTS

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This outline was prepared by F. J. Ludzack, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, Ohio 45268, and revised by C. R. Feldmann, Chemist, National Training Center.

Descriptors: Chemical Analysis, Dissolved Oxygen, Oxygen, Water Analysis

DISSOLVED OXYGEN
DETERMINATION BY ELECTRONIC MEASUREMENT

I INTRODUCTION

A Electronic measurement of DO is attractive for several reasons:

1 Electronic methods are more readily adaptable for automated analysis, continuous recording, remote sensing or portability.

2 Application of electronic methods with membrane protection of sensors affords a high degree of interference control.

3 Versatility of the electronic system permits design for a particular measurement, situation or use.

4 Many more determinations per manhour are possible with a minor expenditure of time for calibration.

B Electronic methods of analysis impose certain restrictions upon the analyst to insure that the response does, in fact, indicate the item sought.

1 The ease of reading the indicator tends to produce a false sense of security. Frequent and careful calibrations are essential to establish workability of the apparatus and validity of its response.

2 The use of electronic devices requires a greater degree of competence on the part of the analyst. Understanding of the behavior of oxygen must be supplemented by an understanding of the particular instrument and its behavior during use.

C Definitions

1 Electrochemistry - a branch of chemistry dealing with relationships between electrical and chemical changes.

2 Electronic measurements or electrometric procedures - procedures using the measurement of potential differences as an indicator of reactions taking place at an electrode or plate.

3 Reduction - any process in which one or more electrons are added to an atom or an ion, such as $O_2 + 2e \rightarrow 2O^{=}$ The oxygen has been reduced.

4 Oxidation - any process in which one or more electrons are removed from an atom or an ion, such as $Zn^o - 2e \rightarrow Zn^{+2}$. The zinc has been oxidized.

5 Oxidation - reduction reactions - in a strictly chemical reaction, reduction cannot occur unless an equivalent amount of some oxidizable substance has been oxidized. For example:

$2H_2 + O_2 \rightleftarrows 2H_2O$

$2H_2 - 4e \rightleftarrows 4H^{+1}$ hydrogen oxidized

$O_2 + 4e \rightleftarrows 2O^{-2}$ oxygen reduced

Chemical reduction of oxygen may also be accomplished by electrons supplied to a noble metal electrode by a battery or other energizer.

6 Anode - an electrode at which oxidation of some reactable substance occurs.

7 Cathode - an electrode at which reduction of some reactable substance occurs. For example in I. C. 3, the reduction of oxygen occurs at the cathode.

8 Electrochemical reaction - a reaction involving simultaneous conversion of chemical energy into electrical energy or the reverse. These conversions are

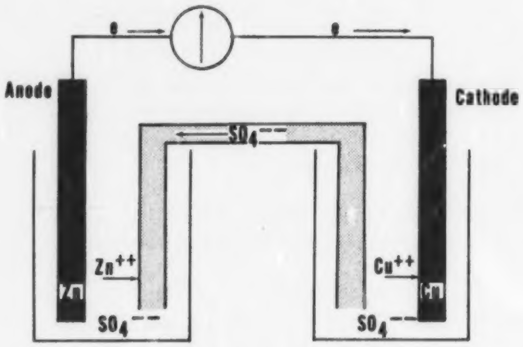
Note: Mention of Commercial Products and Manufacturers Does Not Imply Endorsement by the Environmental Protection Agency.

equivalent in terms of chemical and electrical energy and generally are reversible.

9 Electrolyte a solution, gel, or mixture capable of conducting electrical energy and serving as a reacting media for chemical changes. The electrolyte commonly contains an appropriate concentration of selected mobile ions to promote the desired reactions.

10 Electrochemical cell - a device consisting of an electrolyte in which 2 electrodes are immersed and connected via an external metallic conductor. The electrodes may be in separate compartments connected by tube containing electrolyte to complete the internal circuit.

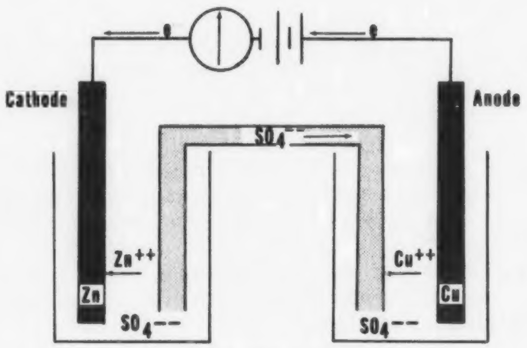
a Galvanic (or voltaic) cell - an electrochemical cell operated in such a way as to produce electrical energy from a chemical change, such as a battery (See Figure 1).



GALVANIC CELL

Figure 1

b Polarographic (electrolytic) cell - an electrochemical cell operated in such a way as to produce a chemical change from electrical energy (See Figure 2).



POLAROGRAPHIC CELL

Figure 2

D As indicated in I. C. 10 the sign of an electrode may change as a result of the operating mode. The conversion by the reactant of primary interest at a given electrode therefore designates terminology for that electrode and operating mode. In electronic oxygen analyzers, the electrode at which oxygen reduction occurs is designated the cathode.

E Each cell type has characteristic advantages and limitations. Both may be used effectively.

1 The galvanic cell depends upon measurement of electrical energy produced as a result of oxygen

reduction. If the oxygen content of the sample is negligible, the measured current is very low and indicator driving force is negligible, therefore response time is longer.

2 The polarographic cell uses a standing current to provide energy for oxygen reduction. The indicator response depends upon a change in the standing current as a result of electrons released during oxygen reduction. Indicator response time therefore is not dependent upon oxygen concentration.

3 Choice may depend upon availability, habit, accessories, or the situation. In each case it is necessary to use care and judgment both in selection and use for the objectives desired.

II ELECTRONIC MEASUREMENT OF DO

A Reduction of oxygen takes place in two steps as shown in the following equations:

1 $O_2 + 2H_2O + 2e \rightarrow H_2O_2 + 2OH^-$

2 $H_2O_2 + 2e^- \rightarrow 2OH^-$

Both equations require electron input to activate reduction of oxygen. The first reaction is more important for electronic DO measurement because it occurs at a potential (voltage) which is below that required to activate reduction of most interfering components (0.3 to 0.8 volts relative to the saturated calomel electrode - SCE). Interferences that may be reduced at or below that required for oxygen usually are present at lower concentrations in water or may be minimized by the use of a selective membrane or other means. When reduction occurs, a definite quantity of electrical energy is produced that is proportional to the quantity of reductant entering the reaction. Resulting current measurements thus are more specific for oxygen reduction.

B Most electronic measurements of oxygen are based upon one of two techniques for evaluating oxygen reduction in line with equation II.A.1. Both require activating energy, both produce a current proportional to the quantity of reacting reductant. The techniques differ in the means of supplying the activating potential; one employs a source of outside energy, the other uses spontaneous energy produced by the electrode pair.

1 The polarographic oxygen sensor relies upon an outside source of potential to activate oxygen reduction. Electron gain by oxygen changes the reference voltage.

a Traditionally, the dropping mercury electrode (DME) has been used for polarographic measurements. Good results have been obtained for DO using the DME but the difficulty of maintaining a constant mercury drop rate, temperature control, and freedom from turbulence makes it impractical for field use.

b Solid electrodes are attractive because greater surface area improves sensitivity. Poisoning of the solid surface electrodes is a recurrent problem. The use of selective membranes over noble metal electrodes has minimized but not eliminated electrode contamination. Feasibility has been improved sufficiently to make this type popular for regular use.

2 Galvanic oxygen electrodes consist of a decomposable anode and a noble metal cathode in a suitable electrolyte to produce activating energy for oxygen reduction (an air cell or battery). Lead is commonly used as the anode because its decomposition potential favors spontaneous reduction of oxygen. The process is continuous as long as lead and oxygen are in contact in the electrolyte and the electrical energy released at the cathode may be dissipated by an outside circuit. The anode may be conserved by limiting oxygen availability. Interrupting the outside circuit may produce erratic behavior for a time after reconnection. The resulting

current produced by oxygen reduction may be converted to oxygen concentration by use of a sensitivity coefficient obtained during calibration. Provision of a pulsed or interrupted signal makes it possible to amplify or control the signal and adjust it for direct reading in terms of oxygen concentration or to compensate for temperature effects.

III ELECTRONIC DO ANALYZER APPLICATION FACTORS

A Polarographic or galvanic DO instruments operate as a result of oxygen partial pressure at the sensor surface to produce a signal characteristic of oxygen reduced at the cathode of some electrode pair. This signal is conveyed to an indicating device with or without modification for sensitivity and temperature or other influences depending upon the instrument capabilities and intended use.

1 Many approaches and refinements have been used to improve workability, applicability, validity, stability and control of variables. Developments are continuing. It is possible to produce a device capable of meeting any reasonable situation, but situations differ.

2 Most commercial DO instruments are designed for use under specified conditions. Some are more versatile than others. Benefits are commonly reflected in the price. It is essential to determine the requirements of the measurement situation and objectives for use. Evaluation of a given instrument in terms of sensitivity, response time, portability, stability, service characteristics, degree of automation, and consistency are used for judgment on a cost/benefit basis to select the most acceptable unit.

B Variables Affecting Electronic DO Measurement

1 Temperature affects the solubility of oxygen, the magnitude of the resulting signal and the permeability of the protective membrane. A curve of oxygen solubility in water versus increasing temperature may be concave downward while a similar curve of sensor response versus temperature is concave upward. Increasing temperature decreases oxygen solubility and increases probe sensitivity and membrane permeability. Thermistor actuated compensation of probe response based upon a linear relationship or average of oxygen solubility and electrode sensitivity is not precisely correct as the maximum spread in curvature occurs at about 17° C with lower deviations from linearity above or below that temperature. If the instrument is calibrated at a temperature within + or - 5° C of working temperature, the compensated readout is likely to be within 2% of the real value. Depending upon probe geometry, the laboratory sensor may require 4 to 6% correction of signal per ° C change in liquid temperature.

2 Increasing pressure tends to increase electrode response by compression and contact effects upon the electrolyte, dissolved gases and electrode surfaces. As long as entrained gases are not contained in the electrolyte or under the membrane, these effects are negligible.

Inclusion of entrained gases results in erratic response that increases with depth of immersion.

3 Electrode sensitivity changes occur as a result of the nature and concentration of contaminants at the electrode surfaces and possible physical chemical or electronic side reactions produced. These may take the form of a physical barrier, internal short, high residual current, or chemical changes in the metal surface. The membrane is intended to allow dissolved gas penetration but to exclude passage of ions or particulates. Apparently some ions or materials producing extraneous ions within the electrode vicinity are able to pass in limited amounts which

become significant in time. Dissolved gases include 1) oxygen, 2) nitrogen, 3) carbon dioxide, 4) hydrogen sulfide, and certain others. Item 4 is likely to be a major problem. Item 3 may produce deposits in alkaline media; most electrolytes are alkaline or tend to become so in line with reaction II.A.1. The usable life of the sensor varies with the type of electrode system, surface area, amount of electrolyte and type, membrane characteristics, nature of the samples to which the system is exposed and the length of exposure. For example, galvanic electrodes used in activated sludge units showed that the time between cleanup was 4 to 6 months for electrodes used for intermittent daily checks of effluent DO; continuous use in the mixed liquor required electrode cleanup in 2 to 4 weeks. Each electrometric cell configuration and operating mode has its own response characteristics. Some are more stable than others. It is necessary to check calibration frequency required under conditions of use as none of them will maintain uniform response indefinitely. Calibration before and after daily use is advisable.

4 Electrolytes may consist of solutions or gels of ionizable materials such as acids, alkalies or salts. Bicarbonates, KCl and KI are frequently used. The electrolyte is the transfer and reaction media, hence, it necessarily becomes contaminated before damage to the electrode surface may occur. Electrolyte concentration, nature, amount and quality affect response time, sensitivity, stability, and specificity of the sensor system. Generally a small quantity of electrolyte gives a shorter response time and higher sensitivity but also may be affected to a greater extent by a given quantity of contaminating substances.

5 Membranes may consist of teflon, polyethylene, rubber, and certain other polymeric films. Thickness may vary from 0.5 to 3 mils (inches × 1/1000). A thinner membrane will decrease response time and increase sensitivity but is less selective and may be ruptured more easily. The choice of material and its uniformity affects response time, selectivity and durability. The area of the membrane and its permeability are directly related to the quantity of transported materials that may produce a signal. The permeability of the membrane material is related to temperature and to residues accummulated on the membrane surface or interior. A cloudy membrane usually indicates deposition and more or less loss of signal.

6 Test media characteristics control the interval of usable life between cleaning and rejuvenation for any type of electrode. More frequent cleanup is essential in low quality waters than for high quality waters. Reduced sulfur compounds are among the more troublesome contaminants. Salinity affects the partial pressure of oxygen at any given temperature. This effect is small compared to most other variables but is significant if salinity changes by more than 500 mg/l.

7 Agitation of the sample in the vicinity of the electrode is important because DO is reduced at the cathode. Under quiescent conditions a gradient in dissolved oxygen content would be established on the sample side of the membrane as well as on the electrode side, resulting in atypical response. The sample should be agitated sufficiently to deliver a representative portion of the main body of the liquid to the outer face of the membrane. It is commonly observed that no agitation will result in a very low or negigible response after a short period of time. Increasing agitation will cause the response to rise gradually until some minimum liquid velocity is reached that will not cause a further increase in response with increased mixing energy. It is important to check mixing velocity to reach a stable high signal that is independent of a reasonable change in sample mixing. Excessive

mixing may create a vortex and expose the sensing surface to air rather than sample liquid. This should be avoided. A linear liquid velocity of about 1 ft/sec at the sensing surface is usually adequate.

8 DO sensor response represents a potential or current signal in the milli-volt or milli-amp range in a high resistance system. A high quality electronic instrument is essential to maintain a usable signal-to-noise ratio. Some of the more common difficulties include:

   a Variable line voltage or low batteries in amplifier power circuits.

   b Substandard or unsteady amplifier or resistor components.

   c Undependable contacts or junctions in the sensor, connecting cables, or instrument control circuits.

   d Inadequately shielded electronic components.

   e Excessive exposure to moisture, fumes or chemicals in the wrong places lead to stray currents, internal shorts or other malfunction.

C Desirable Features in a Portable DO Analyzer

1 The unit should include steady state performance electronic and indicating components in a convenient but sturdy package that is small enough to carry.

2 There should be provisions for addition of special accessories such as bottle or field sensors, agitators, recorders, line extensions, if needed for specific requirements. Such additions should be readily attachable and detachable and maintain good working characteristics.

3 The instrument should include a sensitivity adjustment which upon calibration will provide for direct reading in terms of mg of DO/liter.

4 Temperature compensation and temperature readout should be incorporated.

5 Plug in contacts should be positive, sturdy, readily cleanable and situated to minimize contamination. Water seals should be provided where necessary.

6 The sensor should be suitably designed for the purpose intended in terms of sensitivity, response, stability, and protection during use. It should be easy to clean, and reassemble for use with a minimum loss of service time.

7 Switches, connecting plugs, and contacts preferably should be located on or in the instrument box rather than at the "wet" end of the line near the sensor. Connecting cables should be multiple strand to minimize separate lines. Calibration controls should be convenient but designed so that it is not likely that they will be inadvertently shifted during use.

8 Agitator accessories for bottle use impose special problems because they should be small, self contained, and readily detachable but sturdy enough to give positive agitation and electrical continuity in a wet zone.

9 Major load batteries should be rechargeable or readily replaceable. Line operation should be feasible wherever possible.

10 Service and replacement parts availability are a primary consideration. Drawings, parts identification and trouble shooting memos should be incorporated with applicable operating instructions in the instrument manual in an informative organized form.

D Sensor and Instrument Calibration

The instrument box is likely to have some form of check to verify electronics, battery or other power supply conditions for use. The sensor commonly is not included in this check. A known reference sample used with the instrument in an operating mode is the best available method to compensate for sensor variables under use conditions. It is advisable to calibrate before and after daily use under test conditions. Severe conditions, changes in conditions, or possible damage call for calibrations during the use period. The readout scale is likely to be labeled - calibration is the basis for this label.

The following procedure is recommended:

1 Turn the instrument on and allow it to reach a stable condition. Perform the recommended instrument check as outlined in the operating manual.

2 The instrument check usually includes an electronic zero correction. Check each instrument against the readout scale with the sensor immersed in an agitated solution of sodium sulfite containing sufficient cobalt chloride to catalyze the reaction of sulfite and oxygen. The indicator should stabilize on the zero reading. If it does not, it may be the result of residual or stray currents, internal shorting in the electrode, or membrane rupture. Minor adjustments may be made using the indicator rather than the electronic controls. Serious imbalance requires electrode reconditioning if the electronic check is O.K. Sulfite must be carefully rinsed from the sensor until the readout stabilizes to prevent carry over to the next sample.

3 Fill two DO bottles with replicate samples of clarified water similar to that to be tested. This water should not contain significant test interferences.

4 Determine the DO in one by the azide modification of the iodometric titration.

5 Insert a magnetic stirrer in the other bottle or use a probe agitator. Start agitation after insertion of the sensor assembly and note the point of stabilization.

a Adjust the instrument calibration control if necessary to compare with the titrated DO.

b If sensitivity adjustment is not possible, note the instrument stabilization point and designate it as ua. A sensitivity coefficient, ϕ is equal to $\frac{ua}{DO}$ where DO is the titrated value for the sample on which ua was obtained. An unknown DO then becomes $DO = \frac{ua}{\phi}$. This factor is applicable as long as the sensitivity does not change.

6 Objectives of the test program and the type of instrument influence calibration requirements. Precise work may require calibration at 3 points in the DO range of interest instead of at zero and high range DO. One calibration point frequently may be adequate.

Calibration of a DO sensor in air is a quick test for possible changes in sensor response. The difference in oxygen content of air and of water is too large for air calibration to be satisfactory for precise calibration for use in water.

IV This section reviews characteristics of several sample laboratory instruments. Mention of a specific instrument does not imply EPA endorsement or recommendation. No attempt has been made to include all the available instruments; those described are used to indicate the approach used at one stage of development which may or may not represent the current available model.

A The electrode described by Carrit and Kanwisher (1) is illustrated in Figure 3. This electrode was an early example of those using a membrane. The anode was a silver - silver oxide reference cell with a platinum disc cathode (1-3 cm diameter). The salt bridge consisted of N/2 KCl and

KOH. The polyethylene membrane was held in place by a retaining ring. An applied current was used in a polarographic mode. Temperature effects were relatively large. Thermistor correction was studied but not integrated with early models.

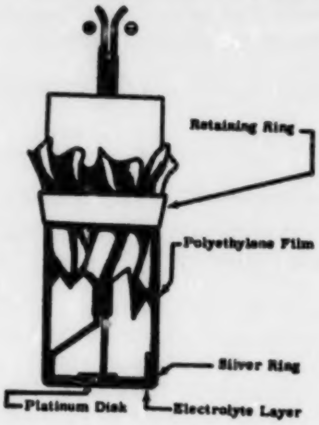

Retaining Ring
Polyethylene Film
Silver Ring
Platinum Disk
Electrolyte Layer

Figure 3

B The Beckman oxygen electrode is another illustration of a polarographic DO sensor (Figure 4). It consists of a gold cathode, a silver anode, an electrolytic gel containing KCl, covered by a teflon membrane. The instrument has a temperature readout and compensating thermistor, a source polarizing current, amplifier with signal adjustment and a readout DO scale with recorder contacts.

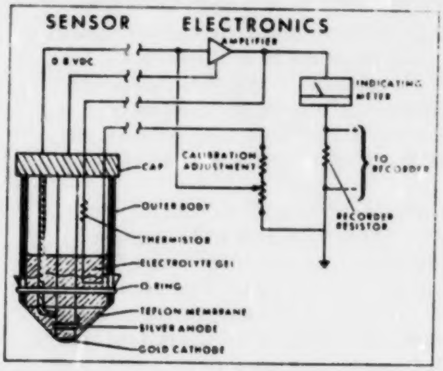

SENSOR
ELECTRONICS
AMPLIFIER
0.8 VDC
INDICATING METER
CALIBRATION ADJUSTMENT
TO RECORDER
RECORDER RESISTOR
CAP
OUTER BODY
THERMISTOR
ELECTROLYTE GEL
O RING
TEFLON MEMBRANE
SILVER ANODE
GOLD CATHODE

Figure 4. THE BECKMAN OXYGEN SENSOR

C The YSI Model 51 (3) is illustrated in Figure 5. This is another form of polarographic DO analyzer. The cell consists of a silver anode coil, a gold ring cathode and a KCl electrolyte with a teflon membrane. The instrument has a sensitivity adjustment, temperature and DO readout. The model 51 A has temperature compensation via manual preset dial. A field probe and bottle probe are available.

YSI Model 51 DO Sensor

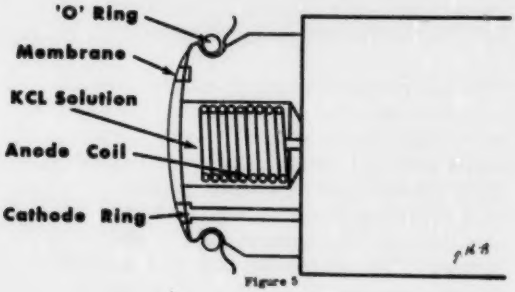

'O' Ring
Membrane
KCL Solution
Anode Coil
Cathode Ring
pKB

Figure 5

D The Model 54 YSI DO analyzer (4) is based upon the same electrode configuration but modified to include automatic temperature compensation, DO readout, and recorder jacks. A motorized agitator bottle probe is available for the Model 54 (Figure 6).

YSI Model 54 Agitator Probe

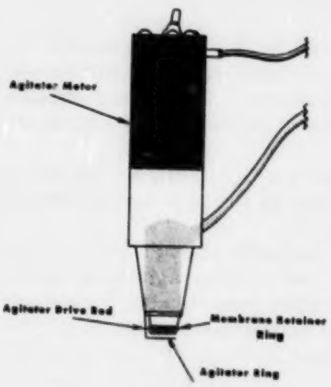

Agitator Motor
Agitator Drive Rod
Membrane Retainer Ring
Agitator Ring

Figure 6

E The Galvanic Cell Oxygen Analyzer (7, 8) employs an indicator for proportional DO signal but does not include thermistor compensation or signal adjustment. Temperature readout is provided. The sensor includes a lead anode ring, and a silver cathode with KOH electrolyte (4 molar) covered by a membrane film (Figure 7).

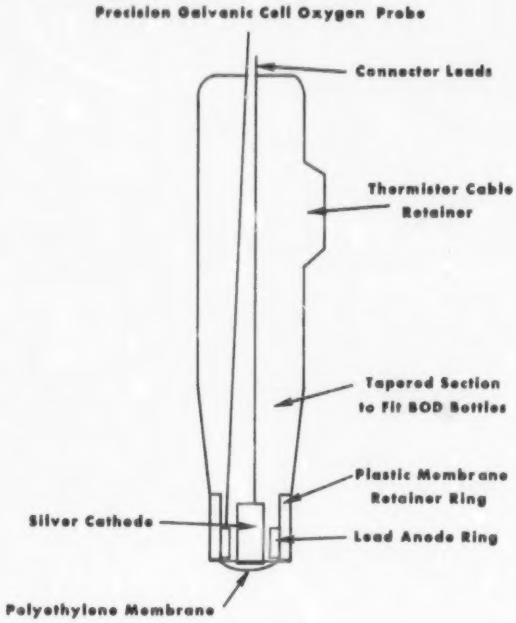

Precision Galvanic Cell Oxygen Probe
Connector Leads
Thermistor Cable Retainer
Tapered Section to Fit BOD Bottles
Plastic Membrane Retainer Ring
Silver Cathode
Lead Anode Ring
Polyethylene Membrane

Figure 7

F The Weston and Stack Model 300 DO Analyzer (8) has a galvanic type sensor with a pulsed current amplifier adjustment to provide for signal and temperature compensation. DO and temperature readout is provided. The main power supply is a rechargeable battery. The sensor (Figure 8) consists of a lead anode coil recessed in the electrolyte cavity (50% KI) with a platinum cathode in the tip. The sensor is covered with a teflon membrane. Membrane retention by rubber band or by a plastic retention ring may be used for the bottle agitator or depth sampler respectively. The thermistor and agitator are mounted in a sleeve that also provides protection for the membrane.

G The EIL Model 15 A sensor is illustrated in Figure 9. This is a galvanic cell with thermistor activated temperature compensation and readout. Signal adjustment is provided. The illustration shows an expanded scheme of the electrode which when assembled compresses into a sensor approximating 5/8 inch diameter and 4 inch length exclusive of the enlargement at the upper end. The anode consists of compressed lead shot in a replaceable capsule (later models used fine lead wire coils), a perforated silver cathode sleeve around the lead is covered by a membrane film. The electrolyte is saturated potassium bicarbonate. The large area of lead surface, silver and membrane provides a current response of 200 to 300 microamperes in oxygen saturated water at 20° C for periods of up to 100 days use (8). The larger electrode displacement favors a scheme described by Eden (9) for successive DO readings for BOD purposes.

V Table 1 summarizes major characteristics of the sample DO analyzers described in Section IV. It must be noted that an ingenious analyst may adapt any one of these for special purposes on a do-it-yourself program. The sample instruments are mainly designed for laboratory or portable field use. Those designed for field monitoring purposes may include similar designs or alternate designs generally employing larger anode, cathode, and electrolyte capacity to approach better response stability with some sacrifice in response time and sensitivity. The electronic controls, recording, telemetering, and accessory apparatus generally are semi-permanent installations of a complex nature.

ACKNOWLEDGMENTS:

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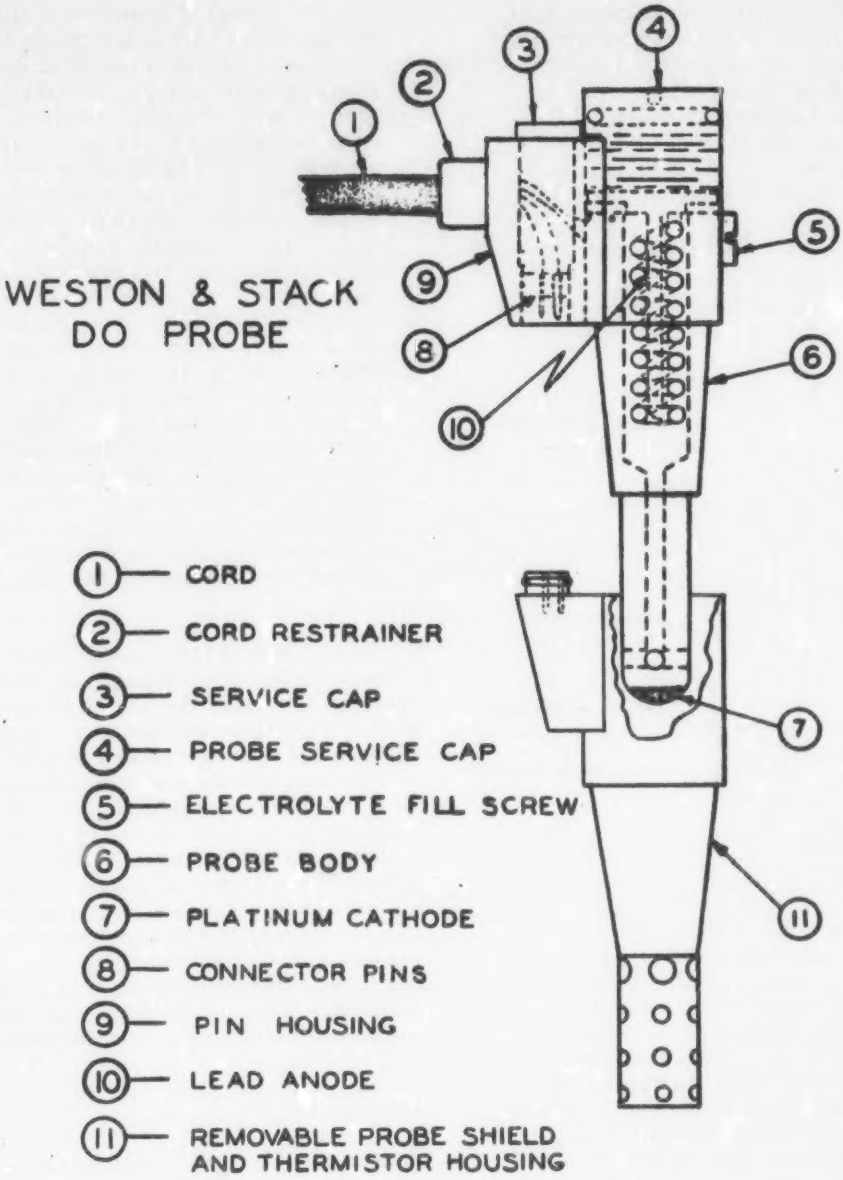
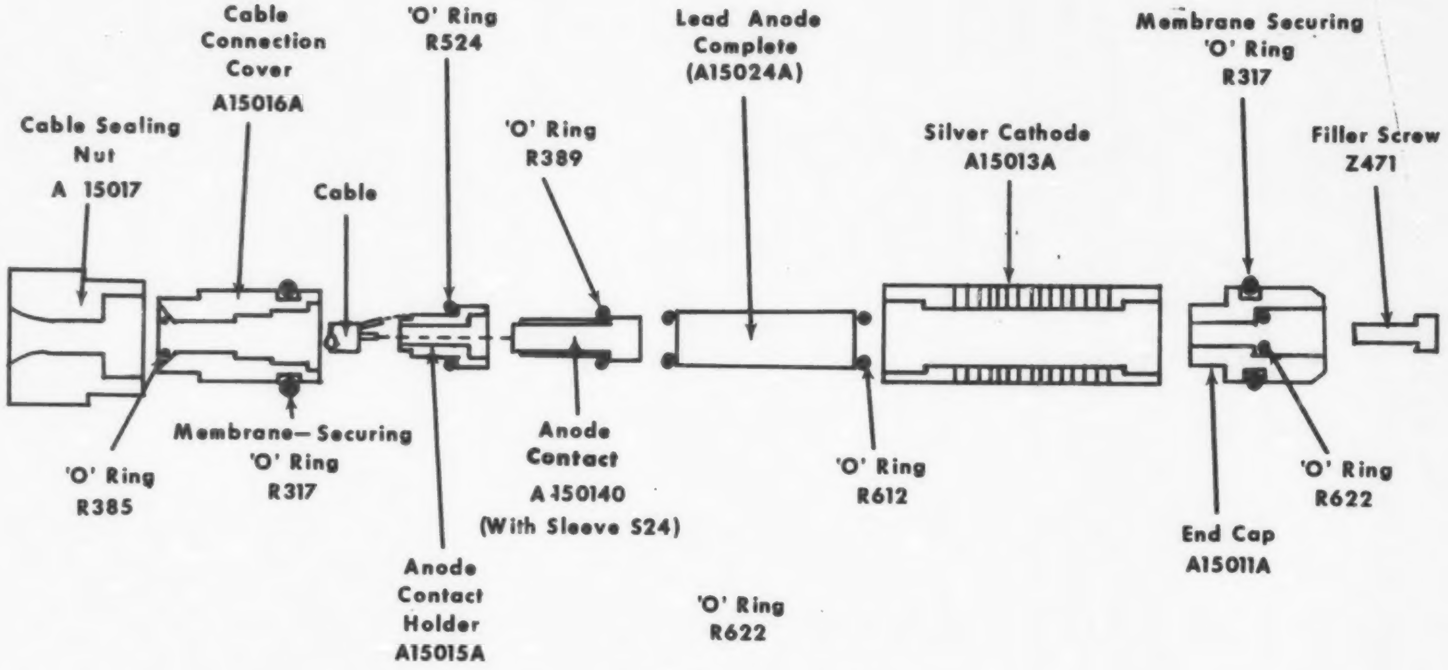

WESTON & STACK
DO PROBE
①
②
③
④
⑤
⑥
⑦
⑧
⑨
⑩
⑪
① CORD
② CORD RESTRAINER
③ SERVICE CAP
④ PROBE SERVICE CAP
⑤ ELECTROLYTE FILL SCREW
⑥ PROBE BODY
⑦ PLATINUM CATHODE
⑧ CONNECTOR PINS
⑨ PIN HOUSING
⑩ LEAD ANODE
⑪ REMOVABLE PROBE SHIELD AND THERMISTOR HOUSING

Figure 8

Model A15A ELECTRODE COMPONENT PARTS


Cable Sealing Nut A 15017
Cable Connection Cover A15016A
Cable
'O' Ring R524
'O' Ring R389
Lead Anode Complete (A15024A)
Silver Cathode A15013A
Membrane Securing 'O' Ring R317
Filler Screw Z471
'O' Ring R385
Membrane—Securing 'O' Ring R317
Anode Contact A 150140 (With Sleeve S24)
Anode Contact Holder A15015A
'O' Ring R612
'O' Ring R622
End Cap A15011A
'O' Ring R622

Note: Red wire of cable connects to Anode Contact Holder

Black wire of cable connects to Anode Contact

Membrane not shown E. I. L. part number T22.

Figure 9

TABLE 1

CHARACTERISTICS OF VARIOUS LABORATORY DO INSTRUMENTS

	Anode	Cathode	Elec	Type	Membr	DO Sig. Adj.	Temp. Comp. Temp. Rdg.	Accessories for which designed
Carrit & Kanwisher	silver-silver ox. ring	Pt disc	KCl KOH N/2	pol*	polyeth	no	no	Recording temp. & signal adj. self assembled
Beckman	Aq ring	Au disc	KCl gel	pol	teflon	yes	yes yes	recording
Yellow Springs 51	Ag coil	Au ring	KCl soln sat.	pol	teflon	yes	no* yes	field and bottle probe
Yellow Springs 54	"	"	"	"	"	yes	yes yes	recording field bottle & agitator probes
Precision Sci	Pb ring	silver disc	KOH 4N	galv**	polyeth	no	no yes	
Weston & Stack 300	Pb coil	Pt disc	KI 40%	galv	teflon	yes	yes yes	agit. probe depth sampler
EIL	Pb	Ag	$KHCO_3$	galv	teflon	yes	yes yes	recording
Delta 75	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes no	field bottle & agitator probe
Delta 85	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes yes	field bottle & agitator probe

*Pol - Polarographic (or amperometric)
**Galv - Galvanic (or voltametric)

REFERENCES

1 Carrit, D.E. and Kanwisher, J.W. Anal. Chem. 31:5. 1959.

2 Beckman Instrument Company. Bulletin 7015, A Dissolved Oxygen Primer, Fullerton, CA. 1962.

3 Instructions for the YSI Model 51 Oxygen Meter, Yellow Springs Instrument Company, Yellow Springs, OH 45387.

4 Instructions for the YSI Model 54 Oxygen Meter, Yellow Springs Instrument Company, Yellow Springs, OH 45387.

5 Technical Bulletin TS-68850 Precision Scientific Company, Chicago, IL 60647.

6 Mancy, K.H., Okun, D.A. and Reilley, C.N. J. Electroanal. Chem. 4:65. 1962.

7 Instruction Bulletin, Weston and Stack Model 300 Oxygen Analyzer. Roy F. Weston, West Chester, PA 19380.

8 Briggs, R. and Viney, M. Design and Performance of Temperature Compensated Electrodes for Oxygen Measurements. Jour. of Sci. Instruments 41:78-83. 1964.

9 Eden, R. E. BOD Determination Using a Dissolved Oxygen Meter. Water Pollution Control. pp. 537-539. 1967.

10. Skoog, D. A. and West, D. M. Fundamentals of Analytical Chemistry. Holt, Rinehart & Winston, Inc. 1966.

11 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL. 1974.

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Descriptors: Chemical Analysis, Dissolved Oxygen, Dissolved Oxygen Analyzers, Instrumentation, On-Site Tests, Water Analysis, Analysis, Wastewater, Oxygen

BIOCHEMICAL OXYGEN DEMAND TEST PROCEDURES

I OXYGEN DEMAND OF POLLUTED WATERS

Established practice includes common use of the BOD test as a tool for estimation of the bio-oxidizable fraction of surface waters or wastewaters discharged to them. Any index including a quantity per unit time such as the BOD_5 is a rate expression. The ultimate demand is more important than any one point on the progression. The results of a bottle test with minimum seeding and quiescent storage are not likely to be as high as those on the same influent in a mixing situation and abundant seed of secondary treatment or receiving waters. The BOD_5 is "a" fraction of total oxygen requirements.

A The particular technique used for BOD commonly is specified by State agencies and/or supervisors. They are required to interpret the results as obtained by laboratory testing. It is essential that the tester and the interpreters have a common understanding of what was done and how. It is highly advisable to maintain a given routine until all concerned agree upon a change.

1 Each particular routine has many undefinable factors. The particular routine is not as important as the consistency and capability with which the result was obtained.

2 This outline and Standard Methods(1) discusses several valid approaches for obtaining BOD results. Selection of "method" is not intended in this outline or in the EPA Methods Manual(2).

B The common 5-day incubation period for BOD testing is a result of tradition and cost. Initial lags are likely to be over and some unknown fraction of the total oxidizable mass has been satisfied after 5 days.

C A series of observations over a period of time makes it possible to estimate the total oxidizable mass and the fraction oxidized or remaining to be oxidized at any given time. The problem is to define the shape of the deoxygenation pattern and its limits. A fair estimate of the shape of the deoxygenation pattern is available by observations at 1, 2, or 3 days, 7 days and 14 days. Increased observations are desirable for more valid estimates of curve shape, rate of oxidation and total oxidizable mass or ultimate BOD.

D Increasing impoundment of surface waters and concurrent increases in complexity and stability of wastewater components emphasize the importance of long-term observation of BOD. The 5-day observation includes most of the readily oxidizable materials but a very small fraction of the stable components that are the main factors in impoundment behavior.

II DIRECT METHOD

A With relatively clean surface waters, the BOD may be determined by incubation of the undiluted sample for the prescribed time interval. This method is applicable only to those waters whose BOD is less than 8 mg/l and assumes the sample contains suitable organisms and accessory nutrients for optimum biological stabilization.

B Treated effluents, polluted surface waters, household and industrial wastewaters commonly require dilution to provide the excess oxygen required for the oxygen demand determination. General guidelines for dilution requirements for a given BOD range in terms of the percent of sample in BOD dilution water are:

For a 5-day BOD of
5-20 mg/l, use 25 to 100% sample

For a BOD of
20-100 mg/l, use 5 to 25% sample

For a BOD of
100-500 mg/l, use 1 to 5% sample

For a BOD of
500-5000 mg/l, use 0.1 to 1.0% sample

III PROCEDURES

A Cylinder Dilution Technique

1 Using an assumed or estimated BOD value as a guide, calculate the factors for a range of dilutions to cover the desired depletions. Those dilutions ranging from a depletion of 2 mg/l and a residual of 1 mg/l are most reliable. At least three dilutions in duplicate should be used for an unknown sample.

2 Into a one-liter graduate cylinder (or larger container if necessary) measure accurately the required amount of mixed sample to give one liter of diluted waste. Fill to the one liter mark with dilution water. Carefully mix. The initial DO by calculation includes IDOD (VIII) a determined initial does not. Both are essential to estimate significance of IDOD. Entrapment of air bubbles during manipulation must be avoided.

3 Siphon the mixture from the cylinder into three 300 ml glass stoppered bottles, filling the bottles to overflowing.

4 Determine the DO concentration on one of the bottles by the appropriate Winkler modification and record as "Initial DO".

5 Incubate the two remaining bottles at 20°C in complete darkness. The incubated bottles should be water-sealed by immersion in a tray or by using a special water-seal bottle.

6 After 5 days of incubation, or other desired interval, determine the DO on the bottles. Average the DO concentration of the duplicates and report as "Final DO".

B Direct Dilution Technique

1 It may be more convenient to make the dilution directly in sample bottles of known capacity. A measured volume of sample may be added (as indicated in A-1) above, and the bottle filled with dilution water to make the desired sample concentration for incubation. In this case, the sample must be precisely measured, the bottle carefully filled, but not overfilled, and the bottle volumes comparable and known. Precision is likely to be poorer than for cylinder dilution.

2 Continue the procedure as in A-4, 5, and 6 above.

C Seeded Cylinder Dilution Technique

1 Many wastewaters may be partially or completely sterile as a result of chlorination, effects of other toxic chemicals, heat, unfavorable pH or other factors detrimental to biological activity. Validity of the BOD result depends upon the presence of organisms capable of prompt and effective biodegradation and favorable conditions during the particular test. Correction of the cause resulting in sterilization must be corrected by adjustment, dilution, etc., prior to reinoculation to achieve meaningful BOD data. Receiving water, biologically treated effluents, and soil suspensions are a good source of organisms likely to be adapted for stabilization of wastewaters. Untreated wastewaters provide numerous organisms but are likely to contain nutrients contributing to excessive seed corrections and may require appreciable time for adaptation before test waste oxidation becomes significant.

2 The amount of added inoculant must be determined by trial. The concentration added should initiate biochemical activity promptly but should not exert enough oxygen demand to unduly reduce the oxygen available for sample requirements.

3 Estimate the sample concentration desired in accordance with A-1 and C-2 above and add the sample aliquot to the dilution cylinder.

4 Add approximately half of the required amount of dilution water to the sample and mix. This is necessary to assure that the concentrated waste does not exert a toxic effect on the seed organisms.

5 Measure a suitable aliquot of seed into the bottle or cylinder and fill with dilution water. Mix the combined sample, seed and dilution water without excessive air entrainment.

6 Continue as in III-A steps 4, 5, and 6 above.

IV INTERPRETATION OF RESULTS

Standard Methods(1) includes a calculation section that is valid and concise. Preceding it are details of reagent preparation and procedures for the test. These will not be reprinted here. This section considers certain items that may cause concern about the validity of results unless they are carefully considered and controlled.

A The initial DO of the BOD test obviously should be high. The method of attaining a high DO can trap the analyst.

1 Aeration of dilution water is the most commonly considered treatment. This technique does produce a high DO but it is a treacherous ally.

a Dirty air passing through clean dilution water can produce clean air and dirty water. This is a simple air-washing operation. Filtering the entering air stream may remove brickbats and 2 x 4's, but filters tend to pass organic gases, fine aerosols, and particulates.

b A stream of air passing through water tends to cool the water by evaporation 1 to 3°C below ambient temperature. The cooled liquid picks up more DO than it can hold at ambient temperature. The physical loss of oxygen may produce an erroneously high depletion value for a determined initial DO, or a low depletion on a calculated initial DO. Erroneous blanks are a particular concern. The dilution water temperature/DO shift is critical.

2 Raising DO by allowing the sample to equilibrate in a cotton-plugged bottle for 2 or 3 days permits oxygenation with minimum air volume contact.

3 Shaking a partially filled bottle for a few seconds also oxygenates with minimum opportunity of gas washing contamination, supersaturation, or temperature changes.

B Seeding always is a precarious procedure but a very necessary one at times. Often the application of seed corrections is a "________ if you do, _______ if you don't" situation. Hopefully, seed corrections are small because each individual biological situation is a "universe" of its own.

1 Unstable seeding materials such as fresh wastewater have "seed" organisms characteristic of their origin and history. Saprophytes resulting in surface water stabilization may be a small fraction of the population. Reactable oxygen-demanding components produce excessive demands upon test oxygen resources.

2 A seed containing viable organisms at a lower energy state because of limited nutritional availability theoretically is the best available seed source. An organism population grown under similar conditions should be most effective for initiating biochemical activity as soon as the nutrient situation favors more activity. The population should not be stored too long because organism redistribution and die-out become limiting. This type of seed would most likely be found in a surface water or a treatment plant effluent with a history of receiving the particular material under consideration.

3 Seed sources and amounts can only be evaluated by trial. Different seed sources and locations require checkout to determine the best available material from a standpoint of rapid initiation of activity, low correction, and predictable high oxygen depletion under test.

C Chlorination and BOD results fundamentally are incompatible. Chlorination objectives include disinfection as the number one goal. Chlorine is notoriously non-specific in organism effects. Chlorine acts like an oxidant in the DO determination. Test organisms are less suitable for activity than they were before chlorination. Nutrients may be less available after chlorination. Certainly the conditions are less suitable for biological response after chlorination. Dechlorination is feasible with respect to the oxidizing power of chlorine, but many organic chlorine compounds that do not show strong oxidizing action still have toxic effects on biologic response.

Numbers are obtainable after dechlorination and reseeding. The meaning of these numbers is obscure. At least two states (New York and New Jersey) specify BOD's before chlorination only.

V PRECISION OF THE BOD TEST

A The DO test precision often has been used to suggest precision of the BOD result.

DO precision is a relatively minor and controllable factor contributing to BOD results. Other factors such as organism suitability, members, adaptation and conditional variables are much more difficult to control or to evaluate.

B The Analytical Reference Service report on Water Oxygen Demand, July, 1960 (Sample type VII) included the results of seeded samples of glucose-glutamic acid BOD results from 34 agencies on 2, 3, 5 and 7 day incubations.

The relative geometric standard deviation (average) was 19% on 2% sample and 24% on 1% sample concentrations. Rate coefficients ranged from 0.10 to 0.27 with a median of 0.16 from 21 different laboratories that participated in rate studies.

VI ALTERNATE BOD TECHNIQUES

Reaeration methods are becoming increasingly popular in order to approach more nearly the actual waste concentration in the receiving water. It is common to obtain "sliding" BOD results related to the concentration of waste in a series of dilutions of the same sample. This may result from greater possibilities for toxic effects at higher concentrations, or to a different selection of organisms and change in oxidation characteristics at low concentrations of sample. The most reliable estimate of stream behavior is likely to be from that dilution closest to the wastewater dilution in the receiving water.

A Reaeration can be accomplished by the usual series techniques by dumping all of the remaining sealed bottles into a common container when the residual DO reaches about 1.0 mg/1. After reoxygenation, the remaining bottles are refilled and a new initial DO determined. Subsequent dissolved oxygen depletions are added incrementally as a summation of the total oxygen depletion from the start of the test. If necessary, the reaeration technique may be performed several times but at a sacrifice of double DO determinations for each day on which reaeration occurs.

B Special methods of reaeration have evolved to minimize the extra manipulation for reaeration of individual sample dilutions.

1 Elmore Method

A relatively large volume of the sample is stored in an unsealed bottle. Small bottles are withdrawn in sets of 5 or more, sealed, incubated, and the DO determined at appropriate intervals. When the DO concentration in the smaller bottles reaches 1.0 mg/1, a new set is withdrawn from the large unsealed bottle, after reoxygenation if necessary.

2 Orford Method

The deoxygenation is carried out in a large sealed jug from which samples for DO are withdrawn at appropriate intervals. To maintain the waste level and a sufficient DO in the jug, additional waste is added from a second open container. See diagram.

C Excess oxygen may be provided by oxygenation with commercial oxygen instead of with air to increase the initial oxygen content for incubation while limiting the number of dilutions or reaeration steps. When oxygen is used in place of air the oxygen saturation in water at 20°C is about 40 mg/1 instead of 9 mg/1. Limited results are available hence the analyst must verify his technique. The DO tends to decrease as soon as the bottle is opened hence, about 35 mg/1 of oxygen content is the top of the practical working concentration. There has been no evidence that the biota is inhibited by the higher oxygen content with respect to BOD progression.

D Reaeration or Oxygenation Advantages and Limitations.

1 Reaeration expands the range of BOD results obtainable directly at field concentrations, but is not advisable for applications when the sample BOD exceeds 50 mg/1.

2 Dilution water problems are eliminated, to the extent that the stream sample may be tested without dilution.

3 Incubator storage space becomes a real problem for multiple sample routine.

VII Dissolved oxygen electrodes, polarographic and others, are feasible for use in BOD determinations, often making it possible to make an estimate of DO or BOD when sample interference prevents a valid Winkler DO determination.

Electronic probe DO makes it possible to determine many successive DO's at different time intervals on the same bottle with negligible sample loss. Reaeration or extended time series, therefore, are more feasible.

Another outline in this series describes response of reaerated BOD_5 with electronic DO probes.

A It is the responsibility of the analyst to evaluate:

1 Applicability of the specified technique and sample.

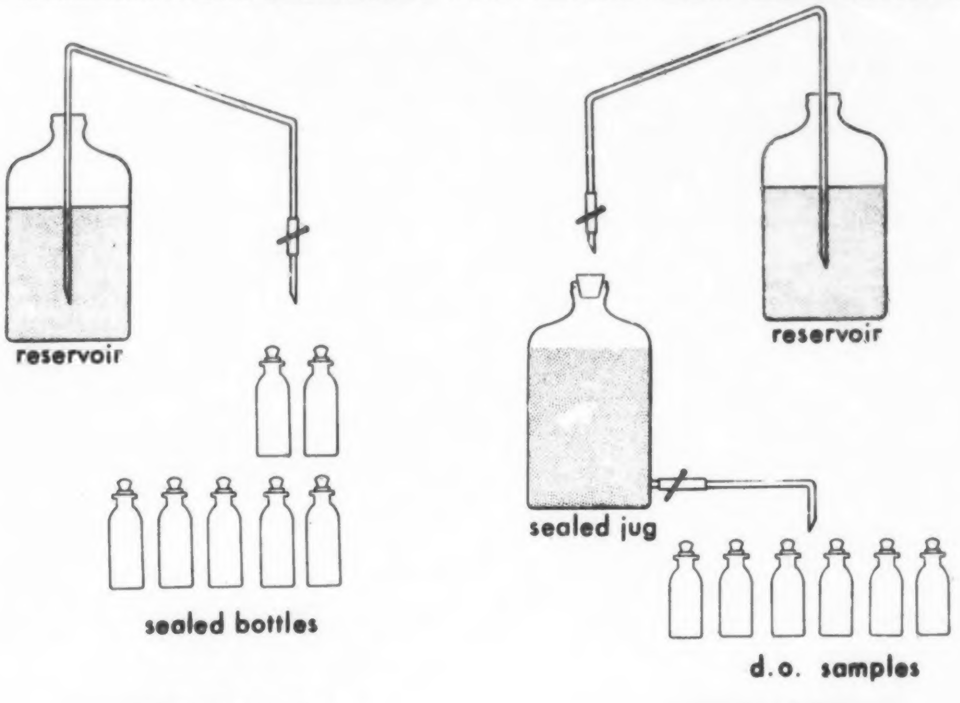
2 To determine requirements for mixing and possible thermal effects while mixing in terms of instrument response and biochemical reaction.

3 To evaluate long-term calibration or standardization and their effects upon precision and accuracy of the BOD result.

VIII IMMEDIATE DISSOLVED OXYGEN DEMAND (IDOD)

Immediate dissolved oxygen demand includes dissolved oxygen utilization requirements of substances such as ferrous iron, sulfite and sulfide which are susceptible to high rate chemical oxidation.

REAERATION METHODS FOR B.O.D. DETERMINATION



ELMORE METHOD

ORFORD METHOD

A The IDOD is an apparent response as indicated by a specified technique. Since DO titration is based upon iodine titration, any factor that causes I_2 response different from that produced by the reaction of KI and molecular oxygen confuses the IDOD determination.

B IDOD Determination

1 The IDOD determination includes the determination of DO on a sample and dilution water separately. A waste likely to have a significant IDOD is unlikely to show a DO.

2 According to mixing theory, it should be possible to calculate the DO of any definite mixture of the sample and dilution water from the DO of component parts and their proportion.

3 The same relative proportions of sample and dilution water should be mixed without air entrainment and the DO determined after the arbitrarily selected time of 15 minutes.

4 Any difference between the calculated initial DO as obtained in 2 above, and the DO determined in 3 above, may be designated as IDOD.

5 Sample aeration, DO interference, and other factors affect results for IDOD.

C Sample Calculation of IDOD

1 Sample DO checked and shown to be 0.0 mg/1

Dilution water DO found to be 8.2 mg/1

Assume a mixture of 9 parts of dilution water and 1 part (V/V) of sample.

Calculated DO =

$1 \times 0 = 0$

$9 \times 8.2 = 73.8$

10 parts of the mixture contain 73.8/10 or 7.4 mg DO/1. Note that mixing has reduced the DO concentration because the original amount is present in a larger package.

2 The mixture described above was held for 15 minutes and the DO determined was 4.3 mg/1.

$$\text{IDOD} = \text{DO}_{\text{calc}} - \text{DO}_{\text{detm}} \times \frac{100}{\%\ \text{sample used}} -$$

$$= 7.4 - 4.3 \times 10$$

$$= 31 \text{ mg IDOD/1}$$

REFERENCES

1 Standard Methods, APHA-AWWA-WPCF 13th Ed. 1971.

2 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL. 1974.

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Descriptors: Biochemical Oxygen Demand, Chemical Analysis, Dissolved Oxygen, Water Analysis, Analysis, Wastewater

BOD DETERMINATION - REAERATED BOTTLE PROBE TECHNIQUE

I INTRODUCTION

A Customary dilution technique has certain limitations with respect to the BOD determination such as a prior commitment on sample dilutions to be used, number of bottles to be included to permit Winkler DO determinations to be made throughout a predetermined test interval, (with sample destruction for each DO test), possibility for anomalous effects due to dilution water, dilution, or inconsistent response between test bottles.

B DO probe technique offers the advantages of nondestructive DO testing, possibilities of adjusting routine according to sample behavior during test, and retaining the sample with minor losses for long term observation (if desired) and for supplementary tests at the end of the BOD test interval.

Note: Use of the probe results in a loss of about 1/2 ml of each 300 ml sample. Use dilution water with a glass rod or bead to bring the sample volume back to 300 ml for further testing.

C This test was performed in triplicate on the same sample at 100% concentration with reaeration as needed to illustrate results obtainable.

II PROCEDURE

A Sample

Final effluent (catch sample at 1100 hours) of the Advanced Waste Treatment Research Activated Sludge (Unit B) Sanitary Engineering Center Experimental Wing (September 3, 1970). One hundred percent concentration.

B DO Probe Calibration

The probe was calibrated daily in air saturated tap water versus the azide modification of the iodometric titration. A replicate test bottle of the titrated sample was retained for initial probe adjustment, if necessary, and to recheck sensor response at the end of the use period. Zero sensor response in cobalt treated sulfite solution was checked at three day intervals. Electronic response and battery condition were checked daily.

C Reaeration

1 When the DO test indicated 1.0 mg DO/liter or appeared likely to reach that point prior to the next test reading the test bottles were reaerated and a new initial DO was obtained.

2 An adapter was inserted into the neck of the sample bottle indicating low DO and an empty bottle inserted on the opposite end. The combination was shaken vigorously, while in an inverted position to allow the transfer of sample to the empty bottle; immediately after transfer the combination was reinverted, shaken as before and sample returned to the original bottle.

3 The sample was allowed to stand for at least five minutes to allow entrained air to rise (generally formed a froth ring). The froth ring was raised into the neck of the bottle by displacement with about 1/2 ml of dilution water. It usually was necessary to tilt the bottle slightly and roll it to sweep fine air bubbles off the shoulder of the bottle and into the neck. After careful reinsertion of the DO sensor these bubbles were displaced by tipping the assembly and discarding contents of the BOD bottle lip containing the frothy residue.

4 It is recognized that surface active materials and possibly other components would concentrate in the discarded froth. It would be possible to limit this effect

by allowing a longer time for the froth to break--say 15 to 30 minutes. This was not attempted for this demonstration.

D No attempt was made to determine DO at any regular time interval. Observation time is recorded for each test on a 24-hour clock basis with zero time at midnight.

E Results are recorded in tabular form including the date (Column 1), time in hours (2), sample temperature (3), hours of incubation (4), and the triplicate sample data. For each of the samples the observed DO, the change since the last observation (ΔDO) and the summation of all the DO depletions observed during the incubated interval are recorded as Σ ΔDO. A bold face line in the tabulated data in the DO column indicates DO before reaeration; the number below the line indicates DO after reaeration to be used as a new initial for the next observation.

F Near the end of the fifth day of incubation (116 hours) one of the triplicates (no. 3) was not reaerated to check the effect of complete depletion of oxygen. It was reaerated on the following day and thereafter.

G The results plotted in graphic form are presented following the BOD tabular data.

H The results of a similar respiration test on mixed liquor from the same activated sludge units are tabulated to show the effects of different types of feed on DO depletion (respiratory activity). In this case, time is in minutes, DO is in mg/l and ΔDO/minute also is in mg/l. The first test in Table 2 represented 100 ml of return sludge plus plant effluent to fill the bottle. Approximately one percent nutrient agar solution was added by syringe (1.0 ml) to produce the response noted in the second series; one ml of five percent mercuric chloride produced the effects in series 3. A new replicate sample (100 ml sludge) and plant influent to fill the bottle produced the results in series 4.

III SUMMARY

A Table 1 shows that it is possible to obtain consistent results using a DO probe plus reaeration for BOD technique. The major requirement is to carefully calibrate the DO instrument and sensor on a daily basis. It is not recommended to reaerate as many times as found necessary here. It could have been diluted at any time since the sample was available. Had the BOD response at five days been substantially complete only two reaerations would have been necessary. However, since the sample was working into second stage BOD it proceeded to react accordingly. The oxygen demand of 15 mg/liter at five days increased to 30 mg/liter in seven days and reached 45 mg/liter in ten days. The sample that was allowed to deplete on the fifth day slowly recovered but by the twelfth day or thereafter equaled or exceeded the BOD of the samples with residual DO at all times. This technique requires much less incubator space, time and manipulation with an added advantage of retaining the sample for adjustment or extended observation. The results do not depend upon a preconceived guestimate which may or may not fit the situation.

B Table 2 indicates the possibility for estimating feed acceptability in an activated sludge treatment plant. On many occasions an inquiry from an industrial plant, a different type of inflow or perhaps a new plant requires a decision regarding effects at the treatment plant. A respiration test by probe technique offers an opportunity to rate the situation within ten minutes after probe calibration. A test of sludge and effluent oxygen demand gives an estimate sludge respiratory activity now. When the same sludge is subjected to a new feed of an acceptable nature respiratory activity rises as in series 2. If the feed happened to be highly toxic as in series 3 (mercuric chloride) respiratory activity stops--

obviously a dead or severely shocked condition. A replicate sludge (Series 4) with the regular plant influent shows a prompt increase in activity. Oxygen requirements, permissible load ratio, acceptable feed to sludge ratios (return sludge adjustment) all may be estimated on the basis of DO tests related to overall plant performance.

C The graphic of BOD versus time includes the COD--an estimate of first stage oxygen demand and the TKN for the composite effluent sample. If the total Kjeldahl nitrogen (TKN) is multiplied by its oxygen equivalent (4.57) then the COD plus TKN times 4.57 gives an estimate of ultimate oxygen demand (138 mg/l).

The twenty day BOD was about 55 × 100/138 or forty percent of this value. The effluent sample obtained at 1100 hours is unlikely to contain a significant portion of the high load period considering the primary, aerator and secondary clarifier detention. Also the COD and TKN may have included materials that were chemically oxidized but gave a poor response to biological oxidation. The BOD gives the rapid respiratory oxygen demand. COD plus the oxygen equivalent of N (on the same sample) gives a quick estimate of potential ultimate demand.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Analysis, Biochemical Oxygen Demand, Chemical Analysis, Dissolved Oxygen, Wastewater, Water Analysis

TABLE 1
BOD Tabulated Results - Reaerated Sample: Probe DO

9/3/70 100% Sample

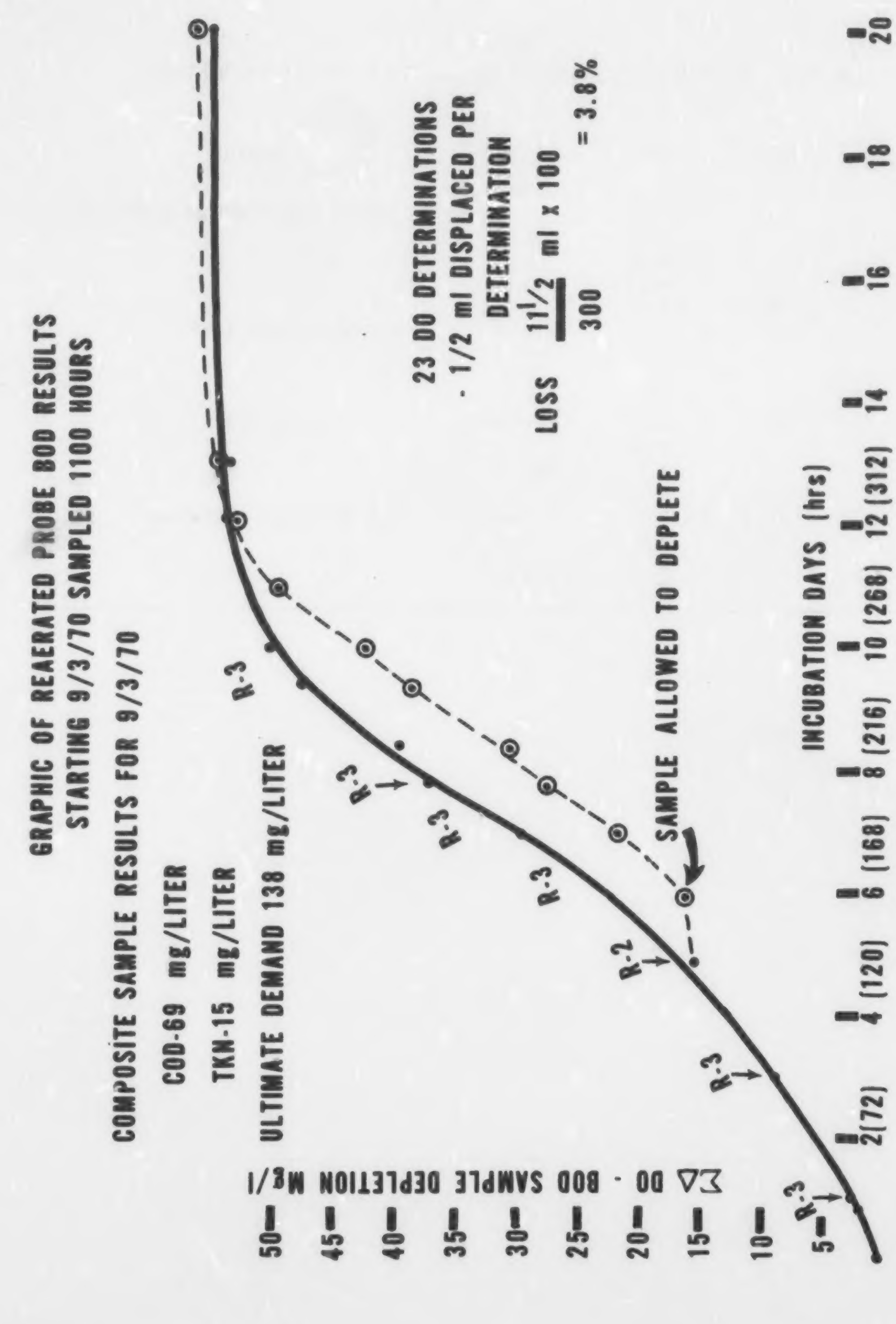
				Sample 1			Sample 2			Sample 3		
Date	Time	T° C	Hrs. Incub	DO	ΔDO	ΣΔDO	DO	ΔDO	ΣΔDO	DO	ΔDO	ΣΔDO
9/3/70	1340	24.0	0	6.7			6.7			6.8		
9/3/70	1355	24.0	0.25	6.7	-		6.7	-		6.8	-	
9/3/70	1620	22.0	2.6	6.5	0.2	0.2	6.5	0.2	0.2	6.6	0.2	0.2
9/4/70	1010	20.2	20.5	4.9 8.2	1.6	1.8	4.7 8.3	1.8	2.0	4.8 7.9	1.8	2.0
9/4/70	1600	20.6	26.3	7.4	0.8	2.6	7.5	0.8	2.8	6.9	1.0	3.0
9/6/70	1340	19.5	72	1.0 8.1	6.4	9.0	1.5 8.1	6.0	8.8	1.1 7.4	5.8	8.8
9/8/70	0940	19.8	116	0.6 8.6	7.5	16.5	0.5 7.6	7.6	16.4	0.4	7.0	15.8
9/9/70	1235	20.0	143	1.1 8.2	7.5	24.0	1.1 8.2	6.5	22.9	0.0 7.6	0.4	16.2
9/10/70	1200	20.0	166	1.0 8.2	7.2	31.2	1.2 8.6	7.0	29.9	2.0 8.0	5.6	21.8
9/11/70	0800	20.0	186	1.2 8.4	7.0	38.2	1.1 8.3	7.5	37.4	2.0 8.5	6.0	27.8
9/11/70	1400	20.0	192	5.0 8.1	3.4	41.6	5.3 8.4	3.0	40.4	5.7 8.6	2.8	30.6
9/12/70	2035	20.0	222	0.5 8.2	7.6	49.2	0.6 8.2	7.8	48.2	0.4 8.2	8.2	38.8
9/13/70	1130	20.0	238	6.3 8.3	1.9	51.1	3.8 8.4	4.4	52.6	4.6 8.3	3.6	42.4
9/14/70	1200	20.0	262	7.0	1.3	52.4	6.9	1.5	53.1	0.9 8.3	7.4	49.8
9/15/70	1340	19.6	288	5.7	1.3	53.7	6.2	0.7	53.8	4.5 8.2	3.8	53.6
9/16/70	1200	19.3	310	5.1	0.6	54.3	5.8	0.4	54.2	7.2	1.0	54.6
*9/20/70	1240	21.6	480	3.8	1.3	55.6	4.6	1.2	55.4	4.9	2.3	56.9

* Incubator power off

TABLE 2

ACTIVATED SLUDGE RESPIRATION DATA DO PROBE TECHNIQUE

Time Min	Temp	DO	ΔDO	Sample
			Series 1	
0	24°	7.2		100 ml Return Sludge & Effluent
1		6.6	0.6	
2		6.2	0.4	
3		5.6	0.6	
4	24.5	5.1	0.5	
0		4.7	Series 2	+ 1 ml Nutrient agar
1		3.8	0.9	
2		2.7	1.1	
3		1.7	1.0	
0	24.8	4.7	Series 3	+ 1 ml $HgCl_2$ Soln
1		4.8		
2		4.7	Nil	
3		4.7		
0	24.2	5.9	Series 4	100 ml Sludge + Influent
1		4.6	1.3	
2		3.3	1.3	
3		2.0	1.3	


GRAPHIC OF REAERATED PROBE BOD RESULTS
STARTING 9/3/70 SAMPLED 1100 HOURS
COMPOSITE SAMPLE RESULTS FOR 9/3/70
COD-69 mg/LITER
TKN-15 mg/LITER
ULTIMATE DEMAND 138 mg/LITER
ΣΔ DO - BOD SAMPLE DEPLETION Mg/l
50
45
40
35
30
25
20
15
10
5
R-3
R-3
R-2
R-3
R-3
R-3
R-3
R-3
SAMPLE ALLOWED TO DEPLETE
23 DO DETERMINATIONS
· 1/2 ml DISPLACED PER DETERMINATION
LOSS 11 1/2 ml x 100 / 300 = 3.8%
INCUBATION DAYS (hrs)
2(72)
4 (120)
6 (168)
8 (216)
10 (268)
12 (312)
14
16
18
20

EFFECT OF SOME VARIABLES ON THE BOD TEST

I TIME

A The common equation $y_t = L\ (1 - 10^{-kt})$ for BOD relationship indicates time as a variable. The rate coefficient (k_1) indicates that a specific percentage of material initially present (oxygen) will be used during a given time unit. Each successive unit of time has less reactant present initially than the preceding interval, hence a definite precentage decrease results in successively smaller amounts of reactant use per unit of lapsed time. Increasing k_1 results in a larger percentage oxygen use per unit of time and also increases the change in reactant mass among successive time intervals.

B Adney's work for the British Royal Commission cited 5 days passage time from source to the ocean as maximum for English streams. The 8th Report (1909) largely established BOD philosophy including the 5-day interval. At 5 days, initial lags generally have terminated and a substantial fraction of the long-term oxygen demand has been exerted. If only one time interval can be used, 7 days permits better scheduling. Any one time interval is "a" fraction of the total oxygen requirement; this is a poor reference point if we do not know how it arrived. For example, the percentage of oxidizable material stabilized in terms of oxygen use at various rate factors are:

k_1 ($\log_{10}$)	% oxidized in 5 days	K_1 ($\log_e$)
0.05	42%	0.11
0.10	67%	0.23
0.15	84%	0.34
0.25	94%	0.57
0.50	99+%	1.15

This range ($K_1 = 2.3\ k_1$) is commonly encountered in wastewater stabilization with the higher rates characteristic of fresh oxidizable material that is readily converted. The lower coefficients are characteristic of cell mass at later stages of oxidation and of low-rate reactants in general.

C The oxygen utilization at specified intervals of time are required to estimate k_1, and L, the estimate of oxygen use at infinite time. It is common to observe results at equal intervals of time but this is not essential as long as the time intervals are accurately known. The initial time periods are critical as an error of a few hours in time represents a relatively large change in reactant mass in a system at maximum instability. Unequal time periods can be plotted to define the curve from which any given intervals can be selected as desired.

D Increasing impoundment of surface water provides more time for stabilization of relatively inert soluble or suspended pollutants and for organism adaptation to the situation or pollutants. Long term BOD's are essential to indicate changes in the pattern of oxygen demand vs. time. It may be expected that one or more plateaus will be evident in the BOD curve followed by a temporary rise in rate during second stage oxidation or thereafter. Anaerobiosis may cause a rise in rate coefficient after aerobic conditions are re-established. Eventually k_1 stabilizes at very low values.

1 Rate coefficients tend to be difficult to interpret during long term BOD's because of progressive changes and other factors.

  a The relative error of the DO test may be a large fraction of the incremental DO change during low rate periods.

  b Cell mass may agglomerate under quiescent test conditions and decrease nutrient availability.

c It is not likely that recycled nutrients under aerobic test conditions will have as much effect as recycle from anaerobic benthic deposits in a stream.

2 The BOD result tends to underestimate deoxygenation relative to surface water behavior because of interchanges, turbulence, biota, and boundary effects. Reseeding does not occur in a sealed bottle but reseeding is inevitable in a stream or treatment unit.

II TEMPERATURE

A Effect on Oxidation Rate

Temperature is one of the important controlling factors in any biological system. In the BOD reaction, changes in temperature produce acceleration or depression of the rate of oxidation. Figure 1 shows the changes in the value of k at temperatures from 0 - 25°C on a common wastewater.

B Test Temperature

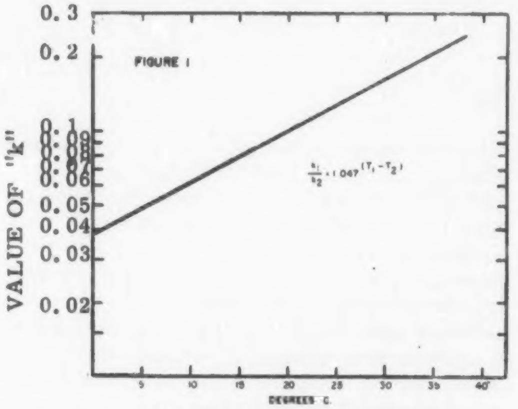
In the BOD test procedure an arbitrary temperature is usually selected for convenience even though a wide temperature range exists under natural conditions.

Incubation of the test containers at 20°C for the whole period is now accepted practice in the U.S.; 18.5°C is preferred in England. Camp (ASCE, SA5 91:1, Oct. 65) recommended light and dark bottle immersion in the stream.

C Temperature Correction

When it is necessary to calculate the rate of oxidation at a temperature other than 20°, the following relationship may be used:

$$\frac{k_1}{k_2} = \theta^{(T_1 - T_2)}$$



VARIATION IN "k" WITH CHANGE IN TEMPERATURE

where:

k_1 = rate coefficient at temperature T_1

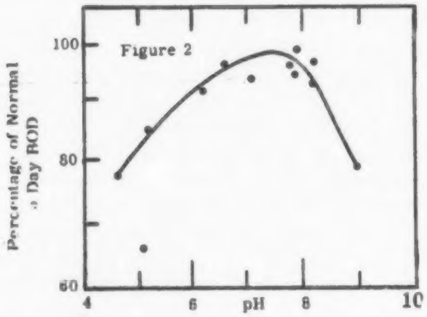
k_2 = rate at coefficient at temperature T_2

θ = temperature coefficient, for which Streeter and Phelps obtained the value 1.047. θ changes with temperature; it appears to be higher in the range of 5-15°C than in the range of 30 to 40°C. The value given refers to 15-30°.

The cited temperature coefficient appears reasonable for household wastes. It may not apply for other wastes where developing or seed organisms may not tolerate temperature changes as readily. A given temperature coefficient should be checked for applicability under specified conditions.

III pH

A The organisms involved in biochemical conversions apparently have an optimum response near a pH of 7.0 providing other environmental factors are favorable; a pH range of about 6.5 to 8.3 apparently is acceptable (Figure 2). Reactivity is likely to be significantly lower on both sides of the acceptable pH range but microbial adaptation may extend the limits appreciably. For example, trickling filters have operated with better than 50% treatment efficiency at pH 3 and 10 after adaptation.



B Adjustment of Concentrated Samples

When wastes are more acid than pH 6.5 or more alkaline than pH 8.3, adjustment to pH 7.2 is advisable before reliable BOD values can be obtained.

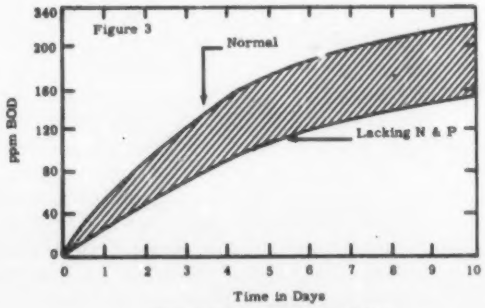
C Dilution Samples

Standard dilution water is buffered at pH 7.2. Sample-dilution water mixtures should be checked to make sure that the sample buffer capacity does not exceed the capacity of the dilution water for pH adjustment.

IV ESSENTIAL MINERAL NUTRIENTS

A Importance

In 1932 Butterfield reported on the role of certain minerals in the biochemical oxidation of sewage and concluded that deficient minerals often upset metabolic response. In addition, he found that inadequate nitrogen and/or phosphorus was a common cause of low BOD results in industrial wastewaters. (Figure 3)



Effect of Mineral Nutrients on BOD

B Standard Methods Dilution Water

The dilution water specified for the BOD test approximates USGS estimates for an average U.S. mineral content of surface water except for added phosphate buffer. It is assumed to provide essential mineral nutrients for most wastewaters but cannot be expected to meet requirements for grossly deficient wastewater nutrients both mineral and organic. Ruchhoft (S.W.J. 13:669, 1941) summarized committee action leading to the present dilution water.

C Other Dilution Considerations

There is a trend toward the use of receiving water, storage-stabilized if necessary, to evaluate waste behavior. It is advisable to minimize dilution and consider the nutrient level likely in the receiving water as most valid. Any change in the environment, such as dilution, upsets the microbial balance and requires adaptive changes.

V MICROBIOLOGICAL POPULATION

A Need for Complex Flora and Fauna

Butterfield, Purdy, and Theriault (Pub. Health Rep. 393, 1931) demonstrated that an isolated species of organisms was not as effective in biological stabilization as a variety of species. Figure 4 summarizes some of their data. Bhatta and Gaudy (ASCE, SA3, 91:63, June 1965) reinvestigated this factor. Many studies have emphasized the need for a mixed biota in the BOD test. It appears that bacteria are capable of varied activities, but all species are not capable of synthesizing all required nutrients. Certain bacterial species may be capable of producing enzymes, amino acids, or growth factors needed for their use and by other species for optimum performance. It has been shown that oxygen demand becomes minimal when some limit of bacterial population has been reached. Predation prevents such an approach to maximum numbers and maintains a continuing bacterial growth and recycle of nutrients among a mixed population. The net effect is a symbiotic relation among mixed organisms tending to enhance the rate of stabilization or utilization of oxygen as in the BOD test.

B Organism Adaptation

1 Early investigations in relation to the BOD test considered domestic wastewaters primarily. The saprophytic organisms involved in stabilization either were present in adequate numbers or quickly multiplied to attain effective populations.

2 The period of adjustment required to shift enzyme production needed to utilize an energy source different from that previously utilized or to shift population variety from that favored by one food to that favored by another food is considered an adaptation period. Dilution, temperature, oxygen tension, pH, nutrient type, inhibitory substances, light and other changes all are common inducements for microbial adaptation. Mutation of organisms may be encountered during adaptation but usually is not a factor.

3 The developments in industry and technology have resulted in discharge of new and more varied wastewater constituents. Microorganisms may adapt themselves to the use of a new substance as an energy source providing the energy and environment are favorable. The receiving stream usually shows development of an adapted microbiota for a new or different discharge constituent within hours, days or weeks after fairly regular discharge. The time for adaptation depends on the nature of the constituent, available energy, tolerance of the organisms, and environmental conditions.

C Seeding

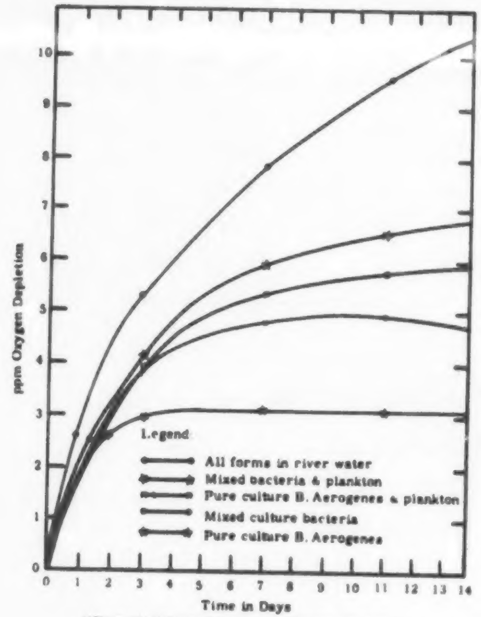
The amount of seed and its selection must be determined experimentally. The most effective inoculant would be that which would produce the maximum BOD response with minimum lag period and negligible seed demand. This would mean some maximum population adapted to feed and conditions at a minimum equilibrium energy nutrient supply.

1 Figure 5 indicates corrected BOD progression on a synthetic feed with river water and stale sewage inoculants at several concentrations. The river water resulted in higher BOD with negligible lag and seed correction. The seed correction at 20% concentration of inoculant was less than 0.3 mg. DO/l at 5 days. It would be possible to use this river water as a diluent without excessive oxygen loss to produce more valid BOD progression for that receiving water. The lower wastewater inoculant concentration resulted in a definite BOD lag. Higher wastewater concentrations produced comparable BOD progression earlier but resulted in high seed corrections and lowered availability of dissolved oxygen for the sample.

2 A good secondary treated effluent produced results similar to river water inoculation with higher seed corrections per increment of applied inoculant. Soil suspensions also are very effective sources of seed organisms with minor seed corrections if they are reasonably stabilized surface soils.

3 It appears that the BOD progression most nearly indicating receiving water oxidation would be one based upon receiving water dilution or inoculated with organisms from it.

4 A new or unusual wastewater may require adapted organisms not present in sufficient numbers in the receiving water. Development of an adapted seed from soil suspensions, plant effluents or receiving water may be necessary to evaluate oxidation potential in a plant or receiving water at some future time. Enrichment culture technique is beneficial where small concentrations of the test wastewater are applied regularly with increases in wastewater concentrations as BOD or respiration activity indicates increasing tolerance and oxidation of the test waste. Both time and concentration limits are useful to characterize the wastewater and its acceptability for biological stabilization.



Effect of Biological Forms on Oxygen Depletion

Figure 4

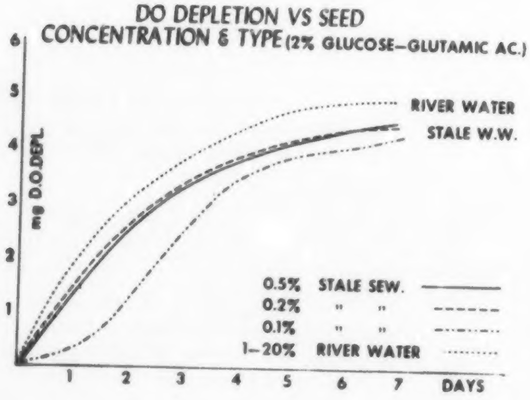


Figure 5

5 It must be recognized that BOD progressions are most likely to err on the low side. A meaningful BOD test should seek the highest consistent oxygen demand feasible for sample and conditions.

D Algae

When large numbers of algae are present in surface waters, they produce significant changes in the oxygen content. Under the influence of sunlight excess oxygen is produced while a net deficit occurs in the dark. The result is a wide variation in surface water DO depending on sample time.

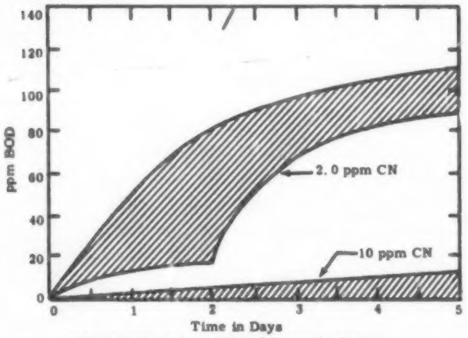
When stream samples containing algae are incubated in the laboratory the algae survive for a time, then die because of the lack of light. Short-term BOD determinations may show the influence of oxygen production by the algae. When the algae die, they release the stored organic load for recycle and increase the BOD. Therefore, samples incubated in the dark may not be representative of the deoxygenation process in the stream, since the benefits of photosynthesis are lacking. Conversely, samples incubated in the light, under conditions of continual photosynthesis, will yield low BOD values.

The influence of algae on BOD is one of the most difficult variables to evaluate. More research is needed to develop satisfactory methods for the accurate determination of BOD in the presence of large numbers of algae. Light and dark bottle incubations suggest the magnitude of effects.

IV TOXICITY

A Effect

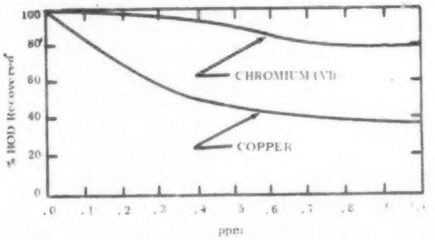
Since satisfaction of the BOD is accomplished through the action of microorganisms, the presence of toxic substances will result in depression of the oxidation rate. In many cases, toxicity will produce a lag period, until tolerant organism activity becomes significant. Figure 6 shows the effect of cyanide on the BOD curve. A prominent lag period is exhibited in the 2 ppm curve, while at 10 ppm the lag extends beyond the fifth day. An activated sludge may be adapted to work effectively in degradation of 60 mg CN/1.



Effect of Cyanide on BOD of Domestic Sewage
(2% Sewage in Formula C Dilution Water)

Figure 6

Heavy metals have similar effects depending on history and environment. The effects of copper and chromium are illustrated in Figure 7.



EFFECT OF HEAVY METALS ON BOD

Figure 7

B Detection

In laboratory determinations of BOD the absence of toxic substances including chlorine must be established before the results can be accepted as valid. Comparison of BOD values for several dilutions of the waste will indicate the presence or absence of toxicity. In Table 1 the calculated BOD for the dilutions show higher values in the more dilute concentrations. It is apparent that toxicity was present and that the toxic effect was diluted out at a waste concentration of 2% or less.

Table I

Waste conc.	Depletion	5 day BOD
10%	3.51	35
5%	4.53	91
2%	2.80	140
1%	1.52	152
0.5%	0.74	148

VII NITRIFICATION

A Mechanism

The oxidation process, as exemplified by the equation:

$$y = L\,(1-10^{-kt})$$

presumably involves the oxidation of carbonaceous matter or 1st stage oxygen demand.

$$C\;H\;O \xrightarrow{O_2} CO_2 + H_2O$$

The rate coefficient is normally high, giving nearly complete oxidation in a few days. When nitrogenous material is present its oxidation can be shown as:

$$NH_3 \xrightarrow{O_2} NO_2^- \xrightarrow{O_2} NO_3^-$$

Nitrogen oxidation may be delayed for several days during BOD tests unless suitable micro-biota are initially available. Under some circumstances these two oxidations can proceed simultaneously and the resultant BOD curve will be a composite of the two reactions.

$$y_t = \left[L_c(1-10^{-k_c t}) + L_n\,(1-10^{-k_n t}) \right]$$

where y_t = the simultaneous BOD of the carbonaceous and nitrogenous phases or 1st and 2nd stage demands.

k_c and k_n = the rate coefficients applicable to the carbonaceous and nitrogenous materials respectively.

L_c and L_n = the ultimate oxygen demands characteristic of the two phases respectively. This is the general formula for any system characterized by two simultaneous reactions.

Principal conditions governing simultaneous carbon and nitrogen oxidation:

1 Presence of an effective nitrifying culture at the beginning of the test interval (nitrifiers grow relatively slowly).

2 Maintenance of adequate DO, believed to be a minimum of 0.5 to 1.0 mg/l, for nitrifier activity.

3 Available nitrogen - in excess of that required for synthesis. This is believed to require a minimum of about 7 mg/l to support active nitrification on a continuous basis.

4 Nitrifiers appear to be more sensitive to toxicity than most saprophytic organisms, hence are likely to be inhibited more readily. This is particularly evident during nitrite to nitrate conversion.

B It may require 5 to 10 days to establish nitrification if the population was not nitrifying initially. This is the basis for the sequential carbonaceous and nitrogenous oxidation of sewage oxidation.

1 Effects on the BOD curve indicate a typical pattern such as in Figure 8. The influence of nitrification in the production of a secondary rise in the BOD curve is so well known that any secondary rise may be erroneously attributed to nitrification whether or not nitrification was involved. Actually, a secondary rise in the curve may be due to any oxidation system assuming dominance after the initial oxidation system has been completed.

2 The nitrification phenomena occurs simultaneously in many streams, treated effluents or partially stabilized samples. The designation of a secondary BOD rise to nitrification should be based on analysis, not curve shape.

C The extent of nitrification is conclusively shown only by periodic analysis of ammonia, organic, nitrite and nitrate nitrogen. The conversion of ammonia and organic nitrogen to oxidized nitrogen is a definite indication of nitrification.

D Nitrification Inhibition

Plant efficiencies from a BOD standpoint can be erroneous because nitrification generally is not established during the usual incubation of influent samples but may be a major factor in effluent incubations. It requires about 2 times the oxygen to convert NH_3 -N to NO_3 -N as to convert C to CO_2 hence this is a major fraction of stream oxygen use. Most secondary treated effluents are characterized by a larger fraction of carbon than nitrogen removal which accentuates the problem.

Pasteurization of samples, methylene blue, chromium, and acid treatment followed by neutralization have been used to inhibit nitrification for estimation of carbonaceous BOD only. Any inhibition of nitrification also produces a change in the sample or its behavior and may partially inhibit carbonaceous oxidation. Nitrification is a factor in stream self-purification and treatment. It does not appear realistic to alter it for convenience. The most realistic approach to carbonaceous oxidation is the measurement of CO_2 or COD.

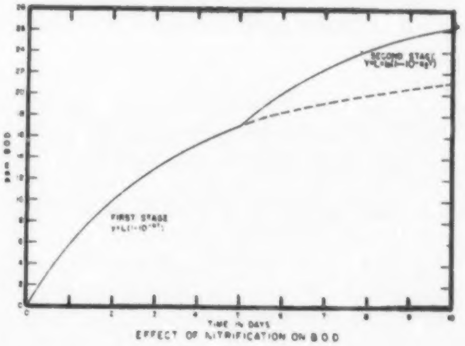


Figure 8

VIII EFFECT OF DILUTION

When a series of dilutions are made on a BOD sample usually the results vary to the extent that only an approximate BOD value is obtained.

Table 2

INTERPRETATION OF BOD DATA

Sample conc.	DO	Depletion	BOD
Initial	8.2	-	-
Final:			
1%	5.5	2.7	270
2%	3.3	4.9	245
4%	0.0	Complete	-

A For example, in Table 2, 1%, 2% and 4% concentrations of sample were used. The 4% concentration became anaerobic before the end of 5 days. The 5-day BOD of the 1% concentration was 270 and that of the 2% concentration was 245.

B Statistically one value is more reliable than the other.

Dilution	DO depletion
1%	5.5 mg/l
2%	3.3 mg/l
Difference	2.2 mg/l

The difference in depletion between 1 and 2% dilutions is 2.2 mg/l. This difference may be attributed to an additional 1% of sample added to the original 1%. If the difference is multiplied by the dilution factor of 100 to obtain the BOD, the result is 220 mg/l.

1 We now have three estimates of the BOD on a one percent concentration basis from the two dilutions:

a the actual 1% depletion gives 270
b 2%/2 depletion gives 245
c (2%- 1%) depletion gives 220

Statistically the probabilities of being nearer the actual value goes with the nearest two of three. The 4% value of 8.2 depletion/4 as a minimum possible BOD 1% concentration gives a BOD of at least 200.

2 There is the possibility that higher concentrations may reflect significant toxicity while lower concentrations tend to reflect a greater proportion of dilution water. The toxicity problem does not appear to be significant since the 4% sample concentration indicated a BOD of at least 200. The higher BOD at 1% sample concentration may be due to a contaminated dilution water or to the fact that a similar number of seed organisms had less food and utilized certain fractions that they had passed by when they had more choice with the 2% sample concentration. Data is insufficient to resolve this one.

3 Incubations having a depletion of at least 2 mg DO/liter and a residual of at least 1 mg DO/liter are indicated to be most valid(1). Both the 1 and 2% concentrations fit this requirement in Table 2. An average error of + or -0.1 ml on the DO titration would have a smaller relative error upon the 2% depletion.

4 We have a reasonable presumption that the sample BOD of about 230 was a good estimate. We do not have an unequivocal basis for so stating. Possible variations in results with different dilutions of a given sample are subject to many uncertainties in the test routine.

If some cause is known - such as a titration eror, the inclusion of extraneous substances producing high or low response, or a definite procedural error that rules out a valid estimate of the sample BOD- that result should be labeled as a lost cause or unreported. Otherwise, report what was obtained to the best of your ability with the provision of uncertainty for uncontrollables.

ACKNOWLEDGMENT:

Certain portions of this outline contain training material from prior outlines by D. G. Ballinger and J. W. Mandia.

REFERENCE:

Standard Methods, APHA-AWWA-WPCF, 13th edition, 1971.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Algae, Ammonia, Bacteria, Biochemical Oxygen Demand, Essential Nutrients, Microorganisms, Nitrates, Nitrification, Nitrites, Nutrients, Oxygen Demand, Rates, Time, Toxicity, Waste Dilution, Water Analysis, Chemical Analysis

MATHEMATICAL BASIS OF THE BIOCHEMICAL OXYGEN DEMAND (BOD) TEST

Part 1

I FUNDAMENTAL CONCEPTS

For a number of years, the oxidation of organic waste substances in surface waters has been under investigation. The first reported observation of oxygen depletion was on the Seine River below Paris (1877). Other studies followed in Germany, England and the U.S. Certain fundamentals are now universally accepted. These are:

A Dissolved oxygen in the water is reduced during stabilization of the organic material.

B As long as dissolved oxygen is present, the rate of oxidation is independent of the actual amount of oxygen available.

C The type and numbers of biological forms present is an important factor.

D Measurement of changes in oxygen content can be related to quantity and character of oxidizable organic matter.

II FIRST ORDER RATE CONCEPT

In order to illustrate the mathematical relationships in the BOD reaction, assume the following laboratory observations:

A A set of replicate bottles is filled with river water and sealed so that outside air is excluded.

B Each day, one of the bottles is analyzed for DO content, and the results tabulated. (See Table 1.)

It will be noted that on each successive day, the DO concentration is less than the day before. That is, oxygen is being consumed by biological or chemical action in the water. The oxygen residual when plotted against time forms the deoxygenation curve (Figure 1).

The oxygen demand or BOD curve is represented by the summation of observed oxygen depletions vs time (Figure 2). This

Table 1.

Days	DO	Oxygen depletion Per day	Oxygen depletion Cumulative
0	9.2	0	-
1	7.4	1.8	1.8
2	5.8	1.6	3.4
3	4.6	1.2	4.6
4	3.7	0.9	5.5
5	2.9	0.8	6.3
6	2.3	0.6	6.9
7	1.8	0.5	7.4
8	1.5	0.3	7.7
9	1.2	0.3	8.0
10	0.9	0.3	8.3

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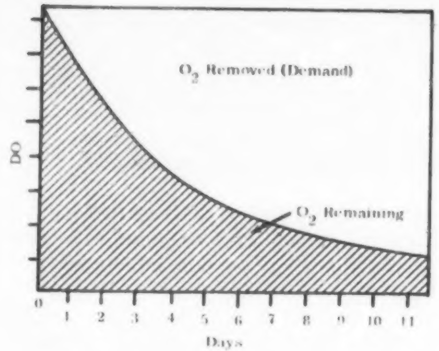


Figure 1

is an inverted representation of the deoxygenation curve. Inclusion of the daily equilavents of oxygen demand in Figure 2 shows that increasingly smaller amounts of oxygen normally are used during each successive day.

If the reaction follows a 1st-order rate system, a constant percentage of the oxygen present at the beginning of each day will be used during that day. Therefore, the rate coefficient (K in nat. log. or k_1 in $\log_{10}$) is constant from day to day. $K_1 = 2.3\ k_1$.

Similarly, the organic matter present in the sample is being oxidized, so there is progressively less oxidizable material present each successive day. The relationship between oxygen demand and the amount of oxidizable material present can be stated as follows: The oxygen demand per unit time is proportional to the amount of unoxidized material present. Streeter and Phelps(1, 2) stated the concept as - the rate of biochemical oxidation of organic matter is proportional to the concentration of unoxidized substance, measured in terms of oxidizability. Such a rate is termed a First Order Reaction Rate. The same authors also stated that there is no logical reason to expect one rate coefficient but it may appear so because of crude measurement and the effects of many individual oxidation systems.

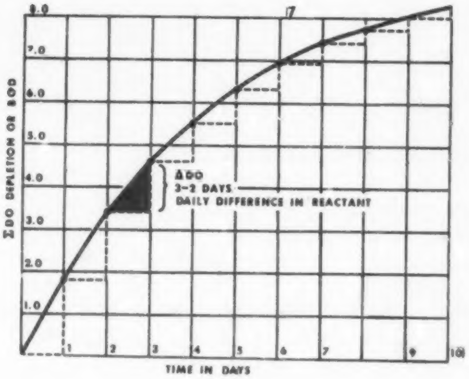


Figure 2. INTEGRAL BOD CURVE

The curve as shown in Figure 2 is a typical BOD curve. When interpreted in light of the fundamentals previously discussed, it is apparent that:

1 With organic materials which are easily oxidized, the reaction proceeds rapidly, nearing stabilization in a few days. The curve is therefore steep, rapidly approaching a maximum.

2 As oxidation nears completion the BOD curve will approach a limit or maximum value that can be represented by a line above the time axis and parallel to it. This line represents the ultimate oxygen demand (L) for the sample and conditions of the test. The curvature of the BOD line as it approaches the maximum is a function of the rate of oxidation.

3 The BOD test commonly is applied for samples containing mixed components each of which may differ in availability as a nutrient. The population of organisms generally contains many varieties. A progression of varieties occurs from those favored by an excess of initial nutrients to those favored by cell mass and eventually to those tolerating a low available energy level.

4 This type of system may have apparent characteristics of 1st order reaction mechanisms for limited periods of time. It is likely that two or more 1st order mechanisms may be apparent if observation time, nutrients and conditions are appropriate. The data also may be interpreted as a zero order, 2nd order or multiple order reaction mechanism. It is not always possible to prove reaction order nor is it essential to do so. The main objectives are to be able to estimate the ultimate BOD, the amount of BOD remaining after a given time interval and to obtain reasonable agreement among observed and calculated data. The first order reaction concept provides "a" means to meet the need without implying that the reactions are necessarily 1st order or that it is the only means of approach.

III BOD EQUATION

Using the typical BOD reaction curve, it is possible to develop equations expressing the various relationships. Labeling the coordinates as in Figure 3.

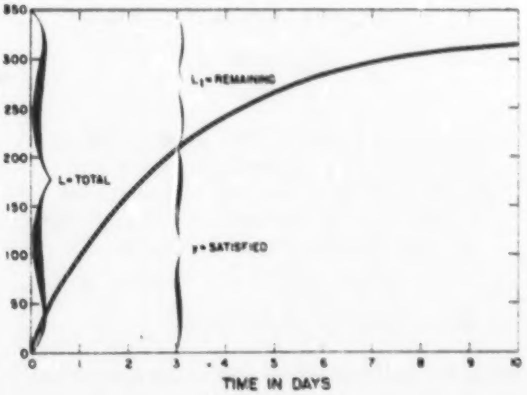


Figure 3

t = time in days

L = ultimate demand

L_t = demand remaining at time t

y = demand satisfied at time t

then:

$\frac{L_t}{L}$ = fraction of L remaining at time t

$\frac{y}{L}$ = fraction of L oxidized at time t

The relationships may be expressed as a differential equation with respect to "t"

$$\frac{dL}{dt} = -KL \qquad (1)$$

where K is a rate coefficient for deoxygenation as a $\log_e$.

$$\text{Ln}\,\frac{(L - y)}{L} = -Kt \qquad (2)$$

changing to $\log_{10}$

$$\text{Log}_{10}\,\frac{(L-y)}{L} = -kt \qquad (3)$$

and:

$$\frac{(L - y)}{L} = 10^{-kt}$$

Table 2
Equivalents in the BOD Equation

BOD remaining at time t	$L-y$	L_t	
Fraction of BOD remaining	$L-y/_L$	$L_t/_L$	10^{-kt}
Conversion of $\log_e$ to $\log_{10}$	K	2.3k	
Fraction of BOD satisfied	$1-\frac{L-y}{L}$	$1-\frac{Lt}{L}$	$1-10^{-kt}$

from Table 2

$$1 - \frac{L-y}{L} = 1 - 10^{-kt} \quad (4)$$

multiplying both sides of equation (4) by L:

$$L\left(1 - \frac{(L-y)}{L}\right) = L(1 - 10^{-kt})$$

rearranging and cancelling gives:

$$y = L(1 - 10^{-kt}) \quad (5)$$

This is the usual form of the BOD equation. To avoid confusion with other "k" values, the deoxygenation rate is usually written as k_1 and the equation becomes.

$$y = L\,(1 - 10^{-k_1 t}) \quad (6)$$

The components in Figure 5 are as in III where L is the ultimate oxygen demand at infinite time and y refers to the demand satisfied at time t. The term k_1 refers to a "velocity constant" in physical chemistry terminology. Section II B3 indicates factors that result in system changes during progression of the BOD test that may affect the value of k_1. For this reason the term "rate coefficient" is used for k_1 to recognize that k_1 may not be a "constant" except for limited time periods. As given, k_1 is an expression of the slope dL/dt as a log and is related to the rate of oxidation.

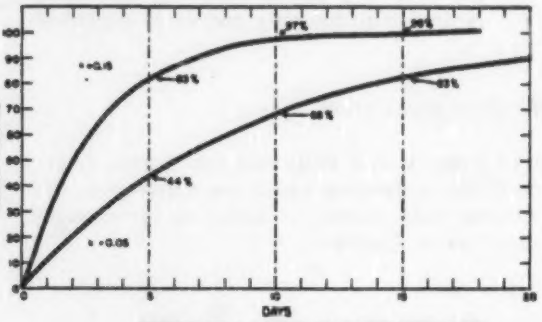
The L value is a laboratory number regarded as a theoretical rather than an actual limit. The limit is useful for an estimation of the effects of a waste upon the receiving water where the total demand is more valid than a part of it as for example a 5 day BOD.

IV SIGNIFICANCE OF BOD CONSTANTS

A The effect of k_1 upon y is shown in Figure 4.

The upper curve represents the BOD of a domestic sewage. The k_1 is 0.15. At this rate, 50% of the demand will be exerted in the first two days, indicating that the organic matter is readily available as food for the organisms. In five days, 83% of the demand has been satisfied and in 15 days, the oxidation is essentially complete.

The lower curve represents the BOD of a relatively stable sample. The k_1 in this case is 0.05. In five days only 44% of the demand is satisfied and at 15 days, there is still 17% of the material unoxidized. Note that at the lower rate, it takes 15 days to accomplish 83% oxidation, while at the higher rate, the same percentage can be accomplished in 5 days.



EFFECT OF K RATE ON SHORT TERM B.O.D.

Figure 4

Laboratory tests on the BOD of waste materials are generally 5-day tests. In the case of these two samples, the standard 5-day interval would represent vastly different degrees of oxidation.

V LOG OF % BOD REMAINING

Using the mathematical relationships established above, a second BOD equation can be developed.

From Table 2:

$$\frac{L - y}{L} = 10^{-k_1 t} = \text{fraction remaining}$$

$$10^{-k_1 t} \times 100 = \%\ \text{remaining}$$

$$\log_{10} (10^{-k_1 t} \times 100) = \log_{10} \%\ \text{remaining}$$

further:

$$\log_{10}(10^{-k_1 t} \times 100) = 2 - k_1 t$$

then:

$$\text{log of \% BOD remaining} = 2 - k_1 t \quad (7)$$

VI SUMMARY

The relationships presented herein form the basis for development of engineering estimates from laboratory data. Validity of the estimate depends upon sampling, prompt and correct BOD technique, judicious data analysis, and realization that the laboratory BOD test does not include many factors controlling stream behavior.

Two examples of the estimation of k and L values are presented in Part 2, which begins on the next page.

ACKNOWLEDGEMENT:

Certain portions of this outline contain materials made available by D. G. Ballinger, F. P. Nixon and D. E. Baumgartner.

REFERENCES

1 Streeter, H. W., and Phelps, E. B. Public Health Bulletin 46, USPHS. 1925.

2 Phelps, E. B. Stream Sanitation. John Wiley & Sons, New York. 1944.

MATHEMATICAL BASIS OF THE BIOCHEMICAL OXYGEN DEMAND (BOD) TEST

ESTIMATION OF k and L

Part 2

I Several methods are available that may give a fair estimate of k_1 and L providing that the observed data plots in a form that suggests a first order reaction rate fit. The fact that stream samples frequently consist of partially stabilized river water mixed with varying amounts of more recent wastewater additions leads to numerous situations where observed data calculated as "a" first order reaction does not give a good fit of observed and calculated data. The low rate oxidation of the aged material and high rate oxidation of the new material simply cannot be explained in terms of one k_1 and L.

The BOD test is not precise enough to distinguish the infinite number of individual rate systems included in deoxygenation of a mixed wastewater by the mixed organisms involved. The persistence of the one first order reaction rate concept (incorrectly called a monomolecular rate) partially is due to the practical limitations of the test. High assimilative oxidation rates generally have been partially completed in the sewer, during compositing or sample storage before BOD analysis, or in the receiving water. Remaining deoxygenation may take the form characteristic of cell mass and storage products, (endogenous oxidation) which for a few days shows a k_1 of about 0.1. If the observation period was extended beyond the usual 7 to 10 days for rate estimation a progressive change of k_1 would become apparent as the more oxidizable components disappear and relatively inert biological or other residues became a larger and larger fraction of the total oxidizable mass. The increased impoundment of surface water makes long-term deoxygenation more and more significant.

The computed k_1 and L are a result of the selected measurement routine, past history of the sample, and the manner of processing the result. The validity of the estimate depends upon engineering judgment and a realization of the variables and effects involved. No two individuals will derive precisely the same values from given data but common sense approaches using mathematics as a tool rather than as a deity, will produce useful estimates for resolution of the problem.

II Useful methods of deriving k_1 include the least squares method of Reed and Theriault (Public Health Bulletin 127, 1927); the Thomas Slope Method (Sew. Works J. 9, No. 3, 425, 1937), the Moments Method of Moore, Thomas, and Snow (Sew. and Ind. Wastes 22, No. 10, 1343, 1950) and the Rapid Ratio Method of Sheehy (JWPCF 32, No. 6, 646, 1960). The first three of these presume a singe rate coefficient and do not give a good fit of data when deoxygenation fails to follow the pattern. Certain other factors enter the picture even when the curve apparently follows first order characteristics (Ludzack et al, Sew. and Ind. Wastes 25, No. 8, 875, 1953). Sheehy's procedure elucidates the disappearance of high rate components during early stages and can be very useful for rapid calculations.

The daily difference method outlined by Tsivoglou (Oxygen Relations in Streams, SEC Tech. Report W-58-2, p. 151, 1958) is an adaptation of Fair and of Velz methods and gives a graphic picture of observed data and predominant rate changes with time. The method is rapid, versatile, and gives a great deal of information in a simple package that is readily assembled. This technique was used to illustrate its applicability as "one" means of estimation.

A Using the data given in Table 1, Part 1.

1 Plot y vs. t on cartisian coordinate paper. Draw a curve of best fit through the observed points, including lags, plateaus, if any, to get a picture of the deoxygenation curve. If a majority of observed values describe a smooth

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progression, those that fail to fit may be considered erratic and curve values may be used for subsequent operations. Lags should be eliminated by curve fitting and taking the observed points after the lag termination. (Figure 2)

2 Plot the daily differences, corrected if necessary, on semilog paper with time on the linear scale and the daily difference on the log scale. The differences are conventionally plotted as 1/2, 1-1/2, 2-1/2 days, etc. to illustrate intervals rather than points. (Figure 5)

3 Examination of Figure 2 shows identical daily differences for the last 3 days or $k_1 = 0$ for that interval in Figure 5. That is unlikely considering the short period of observation and the results of the first 6 days.

4 It may be assumed that deoxygenation follows a smooth curve after considering possible lag periods or multicomponent rates operating in combination or sequentially. Daily differences, therefore, should be calculated from a line of best fit such as Figure 3 rather than from the individual points included in Table 1 which may be affected by experimental error.

5 Figure 5 is a plot of the daily differences from Table 1 on the $\log_{10}$ scale vs time on the arithmetic scale. The corrected curve of best fit was not used here to show the effects of the last 3 points on the logarithmic pattern. The slope of the line was well defined on the basis of the first 7 points. Note that the daily differences are plotted at the middle of the time interval, i.e., the deoxygenation occurring from 0 to 1 day appears at the 1/2 day position and that occurring during the second day appears at the 1-1/2 day position.

6 It is advisable to check arithmetic on the work sheet by comparing the sum of the daily differences in Table 1 with that resulting from the line of best fit in Figure 5. In Table 1: $1.8 + 1.6 + 1.2 + \dots + 0.3 = 8.3$; in Figure 5; $1.88 + 1.50 + 1.20 + \dots + 0.26 = 8.4$. Since the difference in sums is 1 part in 84 it is apparent that serious errors did not occur in arithmetic or plotting.

7 From Figure 5, the number at the zero intercept of the daily difference slope is a function of L. Other intercepts are a function of L_t using equivalents in Table 2. The number representing L at 0 time = 2.1; the number representing L_t when t = 10 is 0.23. The percent BOD remaining when t = 10 is $L_t/L \times 100$ or

$$\frac{0.23 \times 100}{2.1} = 10.9 \text{ or } 11\%.$$

8 From equation 7:

Log_{10} of the % BOD remaining $= 2 - k_1 t$

The Log_{10} of 11% = 1.04 and t = 10
then $1.04 = 2 - k_1 \times 10$

or $k_1 = 2 - 1.04/10 = \dfrac{0.96}{10} = 0.096$ or 0.10

9 From equation 6:

$$y_t = L\,(1 - 10^{-k_1 t}) = L\,(1 - \frac{L_t}{L})$$

From Table 1, $y_{10} = 8.3$, and

$(1 - \dfrac{L_t}{L}) = 0.89$ (Step 7)

The estimate of L becomes

$$y_{10} = L\,(1 - \frac{L_t}{L}) \text{ or } 8.3 = L\,(0.89)$$

$$L = \frac{8.3}{0.89} = 9.34 \text{ or } 9.3 \text{ mg BOD/l.}$$

10 The laboratory test gives estimates of the rate coefficient or k_1 of 0.10 and of L or the Ultimate Oxygen Demand of 9.3 mg of BOD per liter of sample. The data approximates a system consisting primarily of the oxidation of partially stabilized cell mass and storage products and plots as a one rate system during the observation period.

Equations 6 and 7 provide the fundamental relationships and the equivalents in Table 2 may be read from Figure 5.

B The data in Table 3 present a different situation involving more recent contamination.

Table 3

days	BOD	per day
1	2.24	2.24
2	3.18	0.94
3	3.81	0.63
4	4.22	0.41
5	4.56	0.34
6	4.88	0.32
7	5.12	0.24
8	5.32	0.20
9	5.49	0.17
10	5.61	0.12

1 The line of best fit of the observed BODs in Table 3 are shown as a smooth curve in Figure 6.

2 Figure 7 shows the daily differences on the semi log scale vs the time interval on the arithmetic scale. The line ab is a reasonable fit for the slope described by 5 of the 7 daily differences after the 3rd day. It is apparent that the curve in Figure 6 cannot be described as one first order reaction. The differences among the first 3 observed daily differences and the line ab are 1.55, 0.36 and 0.14 from Figure 7. If the differences are plotted on Figure 7, (coded as x) a new rate cd can be estimated that gives a reasonable fit with 2 of the 3 intervals. The first daily difference is above the slope cd suggesting a 3rd rate group higher than ab or cd. Since this would be based upon a single point in 1 series, in which some discrepancy is apparent in Figure 7 with respect to line fitting, it is advisable to regard it as a possibility as shown by the graphic but withhold judgement on the 3rd rate group for further evidence. The curve in Figure 6 may be approximated by a two rate system operating simultaneously with one of the two nearly completed in 3 days.

3 Mathematically this situation may be expressed as:

$$Yt = L_{cd}(1 - 10^{-k_{cd}t}) + L_{ab}(1 - 10^{-k_{ab}t}) \text{ or}$$

$$= L_{cd}\left(1 - \frac{L_t}{L_{cd}}\right) + L_{ab}\left(1 - \frac{L_t}{L_{ab}}\right)$$

where $\frac{L_t}{L}$ may be obtained from Figure 7 at the designated time intervals for each slope.

4 From Figure 7 the zero and 10 day intercepts of line ab are 0.75 and 0.13.

The $\% \frac{L_t}{L} = \frac{0.13 \times 100}{0.75} = 17.4\%$ BOD remaining when $t = 10$.

The $\log_{10}$ of 17.4 is 1.24.

If the $\log_{10}$ of the % BOD remaining $= 2 - k_1 t$

then, $1.24 = 2 - k_{ab} \times 10$ and $k_{ab} =$

$$\frac{2 - 1.24}{10} = \frac{0.76}{10} = 0.076$$

5 From Figure 7 the zero and 2 day intercepts of line cd are 1.40 and 0.22.

$$\% \frac{L_t}{L_{cd}} = \frac{0.22 \times 100}{1.40} = 15.7\% \text{ BOD}$$

remaining when $t = 2$. The $\log_{10}$ of 15.7 is 1.20 as in Step 3:

$$k_{cd} = \frac{2 - 1.20}{2} = \frac{0.80}{2} = 0.40$$

6 From the relation in B2, L_{cd} may be approximated by substituting the observed value for the 2 day BOD in Table 2 or 3.18. The fraction of $\frac{L_t}{L_{ab}}$ from zero to 2 days is $\frac{0.53}{0.75}$ or 0.71 and $(1 - \frac{L_t}{L_{ab}}) = 0.29$, therefore the use of the 2 day observed value for line cd is incorrect because $0.29\ L_{ab}$ is included in the observed value. Since L_{ab} is unknown, the first estimate of L_{cd} will be incorrect by the value of $0.29\ L_{ab}$ but may be approximated later.

If $Y_{2cd} = L_{cd}(1 - \frac{L_t}{L_{cd}})$ where $Y_2 = 3.18$ and $\frac{L_t}{L_{cd}} = \frac{0.22}{1.40}$ then a high approximation of L_{cd} becomes:

$$3.18 = L_{cd} \times 0.84$$

$$L_{cd} = \frac{3.18}{0.84} \text{ or } 3.8 \text{ mg BOD/l.}$$

7 A similar approximation of L_{ab} may be set up for $t = 10$ where the observed Y_{10} from Table 2 of 5.6 is corrected by substracting L_{cd} which should be almost complete by 10 days. Y_{10ab} becomes $5.6 - 3.8 = 1.8$ and $\frac{L_t}{L} = \frac{0.13}{0.75}$ or 0.174.

Substituting the appropriate numbers as in step 5:

$$L_{ab} = \frac{1.8}{0.826} = 2.18 \text{ or } 2.2 \text{ mg/l.}$$

8 A better approximation of L_{cd} is now possible assuming that the 2 day observed value included the following:

$$Y_2 = 3.18 - 0.29 \times 2.2 \text{ or } 3.18 - 0.84 = 2.34$$

and

$$L_{cd} = \frac{2.34}{0.84} = 2.9 \text{ mg BOD/l instead of } 3.8$$

A second approximation of L_{ab} would raise the estimate of L_{ab} because $Y_{10ab} = 5.6 - 2.9 = 2.7$ and

$$L_{ab} = \frac{2.7}{0.826} = 3.28 \text{ or } 3.3 \text{ mg BOD/l}$$

and an estimate of the L values of the sample plotted in Figure 6 would be 2.9 + 3.3 or 6.2 mg BOD/ liter.

C The substitution of the derived values for the appropriate and ultimate oxygen demand into the expression given in B_2 should give values comparable to the observed BODs in Table 3 or Figure 6. Further, it should be possible to approximate curves for the individual rate systems to obtain daily vectors, the sum of which would compare with the observed points. For example:

$$Y_t = L_{cd}(1 - 10^{-k_{cd}t}) + L_{ab}(1 - 10^{-k_{ab}t})$$

Where $L_{cd} = 2.9$ and $k_{cd} = 0.40$

$L_{ab} = 3.3$ and $k_{ab} = 0.076$

and $10^{-k_1 t}$ may be approximated by $\frac{L_t}{L}$ from Figure 7. (See Table 2)

1 $Y_1 = 2.9\ (1 - \frac{0.56}{1.40}) + 3.3\ (1 - \frac{0.63}{0.75})$

$= 2.9 \times 0.60 + 3.3 \times 0.16$

$= 1.74 + 0.53 = 2.27.$ observed = 2.24

2 $Y_2 = 2.9\ (1 - \frac{0.22}{1.40}) + 3.3\ (1 - \frac{0.53}{0.75})$

$= 2.4 + 0.9 = 3.3$ observed = 3.18

3 $Y_3 = 2.9\ (1 - \frac{<0.01}{1.40}) + 3.3\ (1 - \frac{0.44}{0.75})$

$= 2.9 + 1.3 = 4.2$ observed = 3.81

4 $Y_5 = 2.9 + 3.3\ (1 - \frac{0.31}{0.75})$

$2.9 + 1.9 = 4.8$ observed 4.56

5 $Y_{10} = 2.9 + 3.3\left(1 - \frac{0.13}{0.75}\right)$

$= 2.9 + 2.7 = 5.6$ observed 5.61

6 These data show a reasonable approximation of the observed BODs by a reverse calculation from the derived rate and ultimate BOD obtained from them. The agreement indicates that approximations made were reasonably correct and that the composite BOD curve in Figure 6 may be described as a 2 rate system as indicated by the BODs in Figure 6. The largest error occurred in calculation of the components for Y_3 at the point in time where the higher rate system was being phased out. Curve fitting, approximations of correction factors for estimation of L, plotting, slide rule and other errors contribute to estimates of k_1 and L in addition to the errors related to the BOD test.

An approximation of k_1 is possible from 2 BOD values obtained at different intervals of time by using the data presented in the Theriault Tables or that given nomographically by the chart on the Rates of BOD Satisfaction.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, MPOD, OWPO, USEPA. Cincinnati, OH 45268.

Descriptors: Analysis, Analytical Techniques, Biochemical Oxygen Demand, Data Handling, Graphical Analysis, Graphical Methods, Mathematical Models, Mathematical Studies, Mathematics

Figure 5. Log_{10} Slope of the Daily Differences from Figure 2

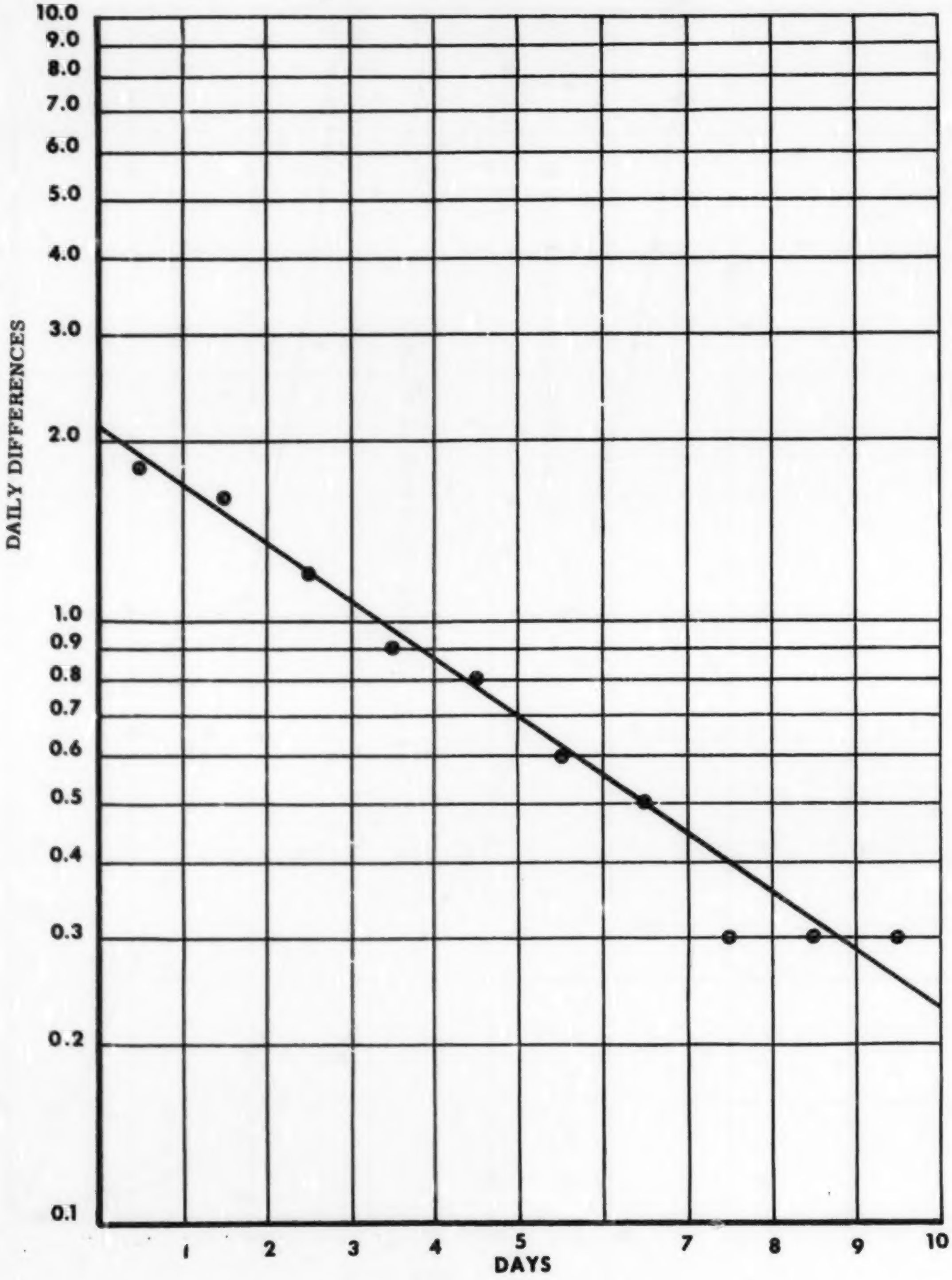


Figure 6. Σ BOD of the Data from Table 2.

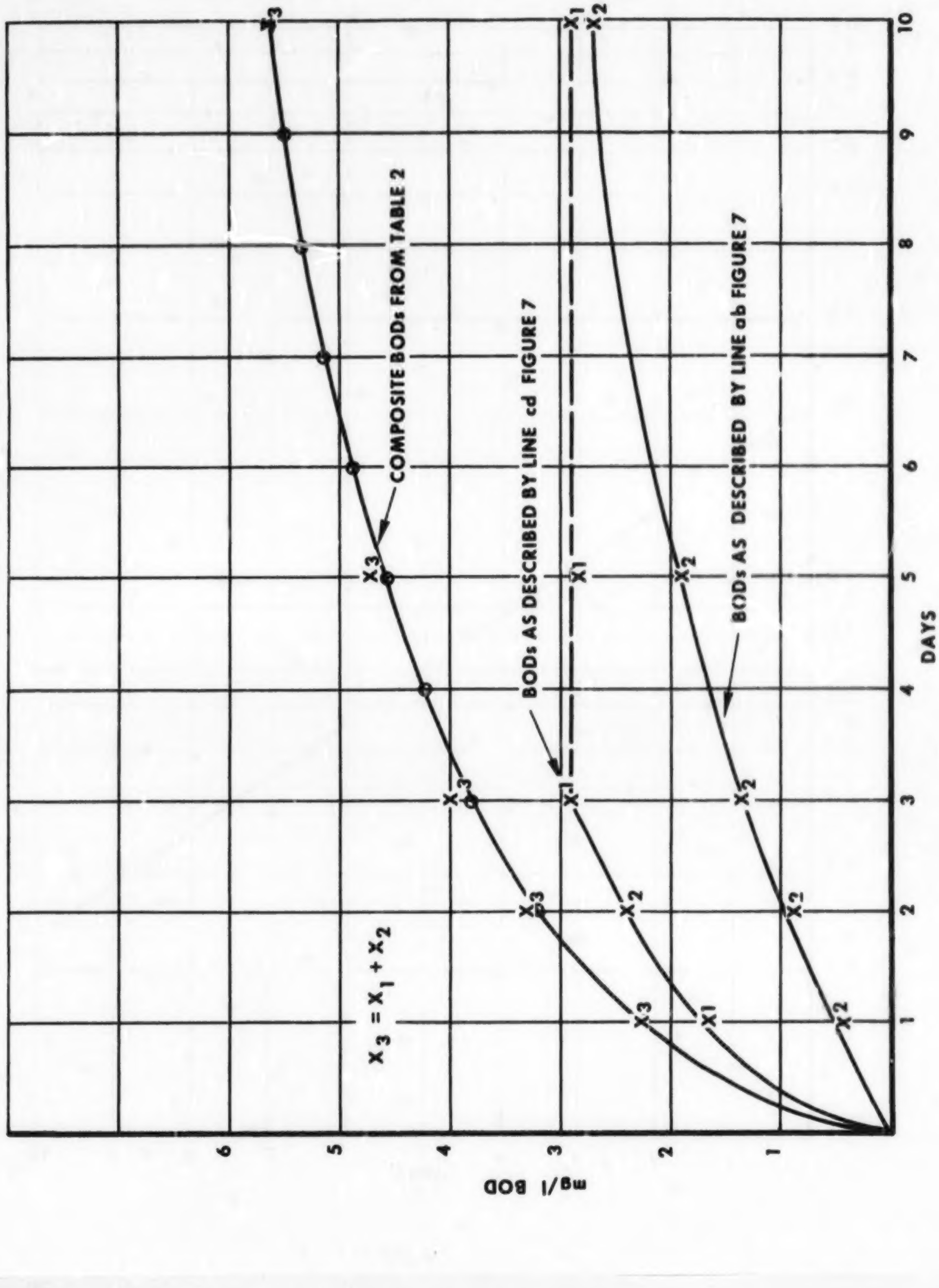
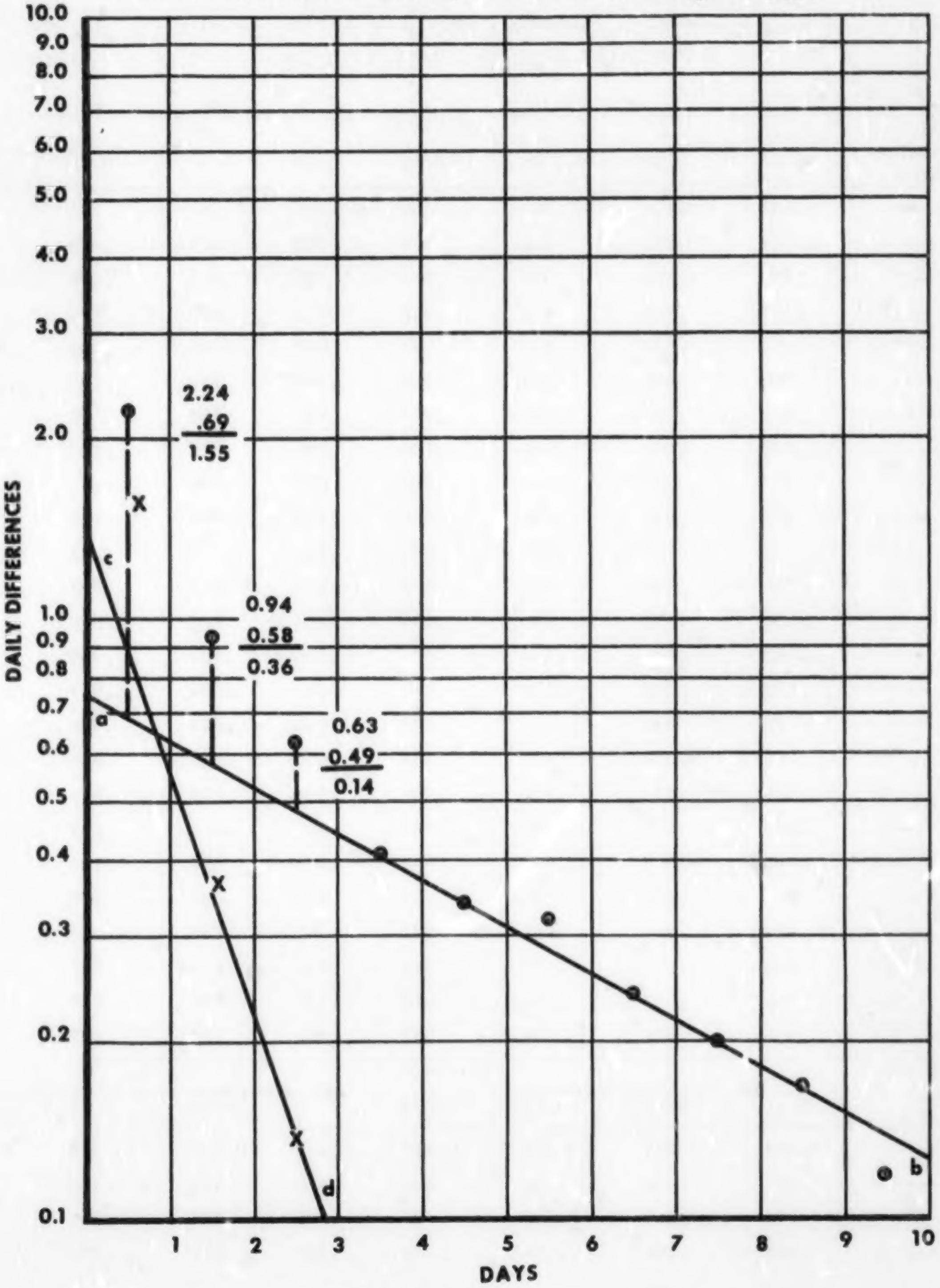


Figure 7. Log_{10} Slopes of the Daily Differences From Figure 6 BODs



Theriault Table

NUMERICAL VALUES OF THE FUNCTION $(1 - 10^{-kt})$ FOR THE RANGE k = 0.040 to k = 0.250

Period of incubation (days)	Value of k						
	0.04	0.05	0.06	0.07	0.08	0.09	0.10
1--------	0.088	0.109	0.129	0.149	0.168	0.187	0.206
2--------	.168	0.206	.241	.276	.308	.339	.369
3--------	.241	0.292	.339	.383	.425	.463	.499
4--------	.308	.369	.425	.475	.521	.563	.602
5--------	.369	.438	.499	.553	.602	.645	.684
6--------	.425	.499	.563	.620	.669	.712	.749
7--------	.475	.553	.620	.676	.725	.766	.800
8--------	.521	.602	.669	.725	.771	.809	.842
9--------	.563	.645	.712	.766	.809	.845	.874
10--------	.602	.684	.749	.800	.842	.874	.900
11--------	.637	.718	.781	.830	.868	.898	.921
12--------	.669	.749	.809	.855	.890	.917	.937
13--------	.698	.776	.834	.877	.909	.932	.950
14--------	.725	.800	.855	.895	.924	.945	.960
15--------	.749	.822	.874	.911	.937	.955	.968
16--------	.771	.842	.890	.924	.948	.964	.975
17--------	.791	.859	.905	.935	.956	.970	.980
18--------	.809	.874	.917	.945	.964	.976	.984
19--------	.826	.888	.928	.953	.970	.981	.987
20--------	.842	.900	.937	.960	.975	.984	.990
21--------	.855	.911	.945	.966	.979	.987	.992
22--------	.868	.921	.952	.971	.983	.990	.994
23--------	.880	.929	.958	.975	.986	.991	.995
24--------	.890	.937	.964	.979	.988	.993	.996
25--------	.900	.944	.968	.982	.990	.994	.997

Period of incubation (days)	Value of k						
	0.11	0.12	0.13	0.14	0.15	0.16	0.17
1--------	0.224	0.241	0.259	0.276	0.292	0.308	0.324
2--------	.397	.425	.450	.475	.499	.521	.543
3--------	.532	.563	.593	.620	.645	.669	.691
4--------	.637	.669	.699	.725	.749	.771	.791

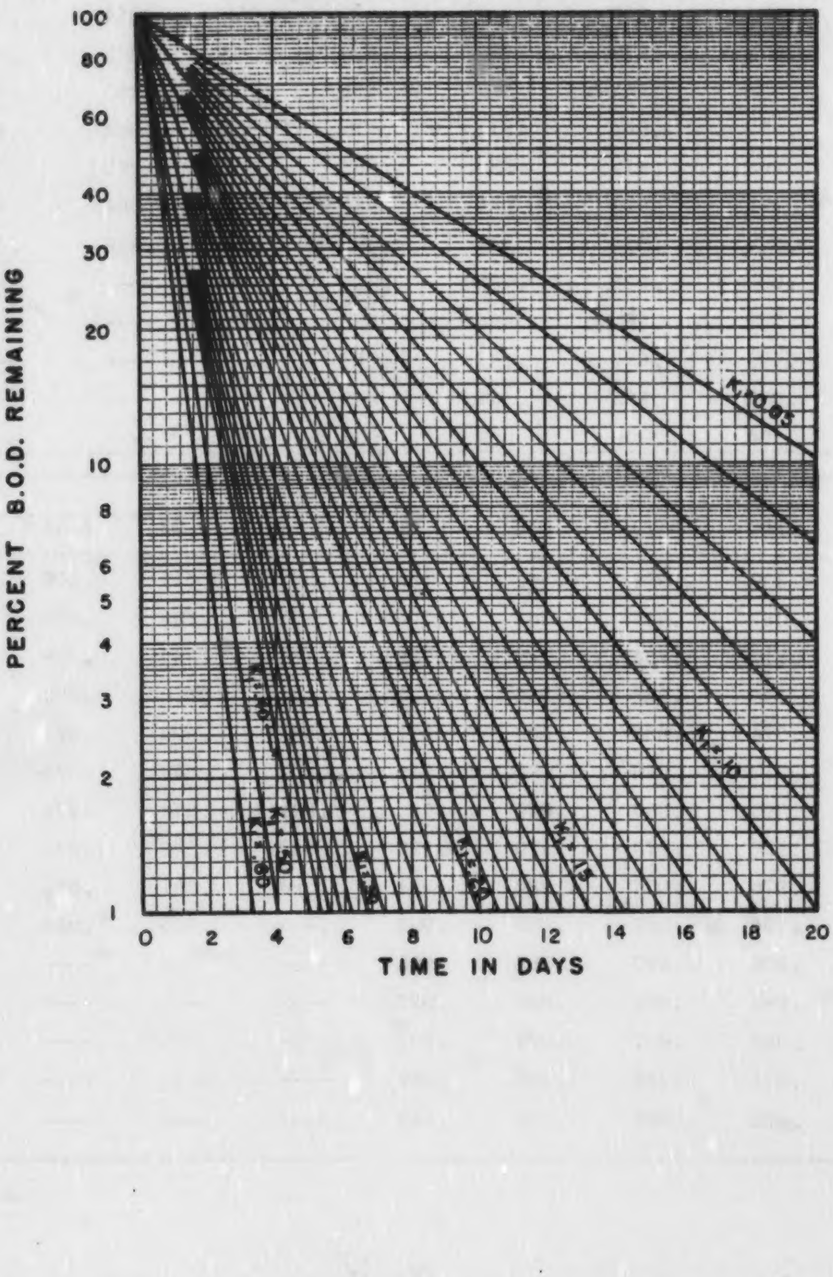
Theriault Table

Period of incubation (days)	Value of k						
	0.11	0.12	0.13	0.14	0.15	0.16	0.17
5--------	.718	.749	.776	.800	.822	.842	.859
6--------	.781	.809	.834	.855	.874	.890	.905
7--------	.830	.855	.877	.895	.911	.924	.935
8--------	.868	.890	.909	.924	.937	.948	.956
9--------	.898	.917	.932	.945	.955	.964	.970
10--------	.921	.937	.950	.960	.968	.975	.980
11--------	.938	.952	.963	.971	.978	.983	.987
12--------	.952	.964	.972	.979	.984	.988	.991
13--------	.963	.972	.980	.985	.989	.992	.994
14--------	.971	.979	.985	.989	.992	.994	.996
15--------	.978	.984	.989	.992	.994	.996	.997
16--------	.983	.988	.992	.994	----	----	----
17--------	.987	.991	.994	.996	----	----	----
18--------	.990	.993	.995	.997	----	----	----
19--------	.992	.995	.997	.998	----	----	----
20--------	.994	.996	.997	.998	----	----	----

Period of incubation (days)	Value of k							
	0.18	0.19	0.20	0.21	0.22	0.23	0.24	0.25
1--------	0.339	0.354	0.369	0.383	0.397	0.411	0.425	0.438
2--------	.563	.583	.602	.620	.637	.653	.669	.684
3--------	.712	.731	.749	.766	.781	.796	.809	.822
4--------	.809	.826	.842	.855	.868	.880	.890	.900
5--------	.874	.888	.900	.911	.921	.929	.937	.944
6--------	.917	.928	.937	.945	.952	.958	.934	.968
7--------	.945	.953	.960	.966	.971	.975	.979	.982
8--------	.964	.970	.975	.979	.983	.986	.988	.990
9--------	.976	.981	.984	.987	.990	.991	.993	.994
10--------	.984	.987	.990	.992	.994	.995	.996	.997
11--------	.990	.992	.994	.995	----	----	----	----
12--------	.993	.995	.996	.997	----	----	----	----
13--------	.995	.997	.997	.998	----	----	----	----
14--------	.997	.998	.998	.999	----	----	----	----
15--------	.998	.999	.999	.999	----	----	----	----

RATES OF B.O.D. SATISFACTION

FOR VALUES OF K_1 FROM .05 TO .60



SOURCES AND ANALYSIS OF ORGANIC NITROGEN

I INTRODUCTION

A Organic nitrogen refers to the nitrogen in combination with any organic radical. For sanitary engineering the main interest is the nitrogen contained in proteins, peptides, amines, amino acids, amides and related compounds of animal or vegetable origin.

II SOURCES OF ORGANIC NITROGEN

A Natural Origin

1 Dead animal and plant residues

2 Animal wastes - urea, feces

3 Autotrophic organisms - algae, s.bact.

4 Heterotrophic organisms

B Industrial Origin

1 Food processing wastewater - meat, milk, vegetables

2 Pharmaceutical wastes, antibiologicals

3 Plastics - polyamides, nitriles

4 Chemical intermediates or products

5 Dye industry - azo, nitro

III TOTAL KJELDAHL NITROGEN PROCEDURE

A Organic nitrogen is determined using the Total Kjeldahl Nitrogen (TKN) method. This determination includes both organic nitrogen and free ammonia. By distilling the free ammonia off the sample before the determination, organic nitrogen can be determined directly.

B Scope

The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia but it may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semi-carbazones and some refractory tertiary amines.

C Method Summary(1)

The sample is heated in the presence of a concentrated sulfuric acid-potassium sulfate-mercuric sulfate mixture and evaporated until sulfur trioxide fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled and diluted, then treated and made alkaline with a hydroxide-thiosulfate solution. The ammonia is distilled off and then determined either by nesslerization or by titration.

1 Nesslerization (colorimetric) is the method used when the ammonia-nitrogen concentration is less than 1.0 mg N/liter.

2 For ammonia-nitrogen concentrations above 1.0 mg N/liter, the ammonia is determined by titration with 0.02 N H_2SO_4 in the presence of a mixed indicator.

For a detailed description of the procedure and reagent preparation, consult the EPA methods manual.

D Precision and Accuracy

1 Thirty-one analysts in 20 laboratories(1) used the Total Kjeldahl Nitrogen procedure to analyze natural water samples containing the following increments of organic nitrogen: 0.20, 0.31, 4.10 and 4.61 mg N/liter.

a Precision results for a standard deviation were 0.197, 0.247, 1.056 and 1.191 mg N/liter, respectively.

b Accuracy as bias was +0.03, +0.02, +0.04 and -0.08 mg N/liter, respectively.

2 The nature and composition of extraneous materials affect analytical recovery. High salt concentrations may raise digestion temperature. High concentrations of organic sample components may require excessive acid during digestion tending to low nitrogen yield.

3 The digestion temperature is critical. 380 to 390°C usually gives high analytical recovery on the more refractory nitrogen compounds of natural origin. Nitrogen losses occur above 420°C.

E Optimum temperature is associated with a digestion mix containing 1 gram of potassium sulfate for each ml of sulfuric acid. However, the digestion mixture usually contains a fraction of H_2SO_4 greater than this.

During heating, the water boils off first, followed by fuming as the H_2SO_4 becomes concentrated. Fuming and oxidation of the sample components lead to loss of H_2SO_4 and a progressive decrease in acid content during heating. As the acid decreases, the temperature of the heated mixture rises.

Valid nitrogen determinations require a slight excess of acid to retain NH_3 as NH_4HSO_4 rather than the more volatile $(NH_4)_2SO_4$. Concurrently, excess acid will tend toward incomplete oxidation of sample components as a result of lowering digestion temperature.

IV AUTOMATED PHENATE METHOD

The EPA manual$^{(1)}$ also presents an automated (phenate) method for organic nitrogen. The sample is automatically digested with a sulfuric acid solution containing potassium sulfate and mercuric sulfate as a catalyst, then neutralized with sodium hydroxide solution and treated with alkaline phenol reagent and sodium hypochlorite reagent. This treatment forms a blue color designated as indophenol. Sodium nitroprusside, which increases the intensity of the color, is added to obtain necessary sensitivity for measurement of low level nitrogen.

V PRESERVATION OF SAMPLES

A Most nitrogen compounds are characterized by rapid conversion from one form to another by biological and chemical action. Hydrolysis, deamination, peptide formation, and other reactions may appreciably alter the original form of sample nitrogen within a short time. Organic nitrogen tends to hydrolyze to free NH_3 during storage. Therefore, valid results require prompt analysis.

B Addition of 2ml of concentrated sulfuric acid or 40 mg $HgCl_2$/liter of sample and storage at 4°C are preservation measures for samples. Even when so preserved, samples are unstable and should be analyzed as soon as possible.

REFERENCE

1 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL. 1974.

This outline was prepared by Audrey E. Donahue, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, OH 45268.

Descriptors: Chemical Analysis, Nitrogen, Nutrients, Water Analysis, Water Pollution Sources

AMMONIA, NITRITES AND NITRATES

I SOURCES AND SIGNIFICANCE OF AMMONIA, NITRITES AND NITRATES IN WATER

The natural occurrence of nitrogen compounds is best demonstrated by the nitrogen cycle (Figure 1).

A Ammonia

1 Occurrence

Ammonia is a product of the microbiological decay of animal and plant protein. In turn, it can be used directly to produce plant protein. Many fertilizers contain ammonia.

2 Significance

The presence of ammonia nitrogen in raw sura surface waters might indicate domestic pollution. Its presence in waters used for drinking purposes may require the addition of large amounts of chlorine in order to produce a free chlorine residual. The chlorine will first react with ammonia to form chloramines before it exerts its full bactericidal effects (free chlorine residual).

B Nitrites

1 Occurrence

Nitrite nitrogen occurs in water as an intermediate stage in the biological decomposition of organic nitrogen. Nitrite formers (nitrosomonas) convert ammonia under aerobic conditions to nitrites. The bacterial reduction of nitrates can also produce nitrites under anaerobic conditions. Nitrite is used as a corrosion inhibitor in industrial process water.

2 Significance

Nitrites are usually not found in surface water to a great extent. The presence of large quantities indicates a source of wastewater pollution.

C Nitrates

1 Occurrence

Nitrate formers convert nitrites under aerobic conditions to nitrates (nitrobacter). During electrical storms, large amounts of nitrogen (N_2) are oxidized to form nitrates. Finally, nitrates can be found in fertilizers.

2 Significance

Nitrates in water usually indicate the final stages of biological stabilization. Nitrate rich effluents discharging into receiving waters can, under proper environmental conditions, cause stress to stream quality by producing algal blooms. Drinking water containing excessive amounts of nitrates can cause infant methemoglobinemia.

II PRESERVATION OF AMMONIA, NITRATE AND NITRITE SAMPLES(8)

A If the sample is to be analyzed for Ammonia or Nitrate, it may be preserved with 2ml concentrated sulfuric acid per liter and cooled to 4°C.

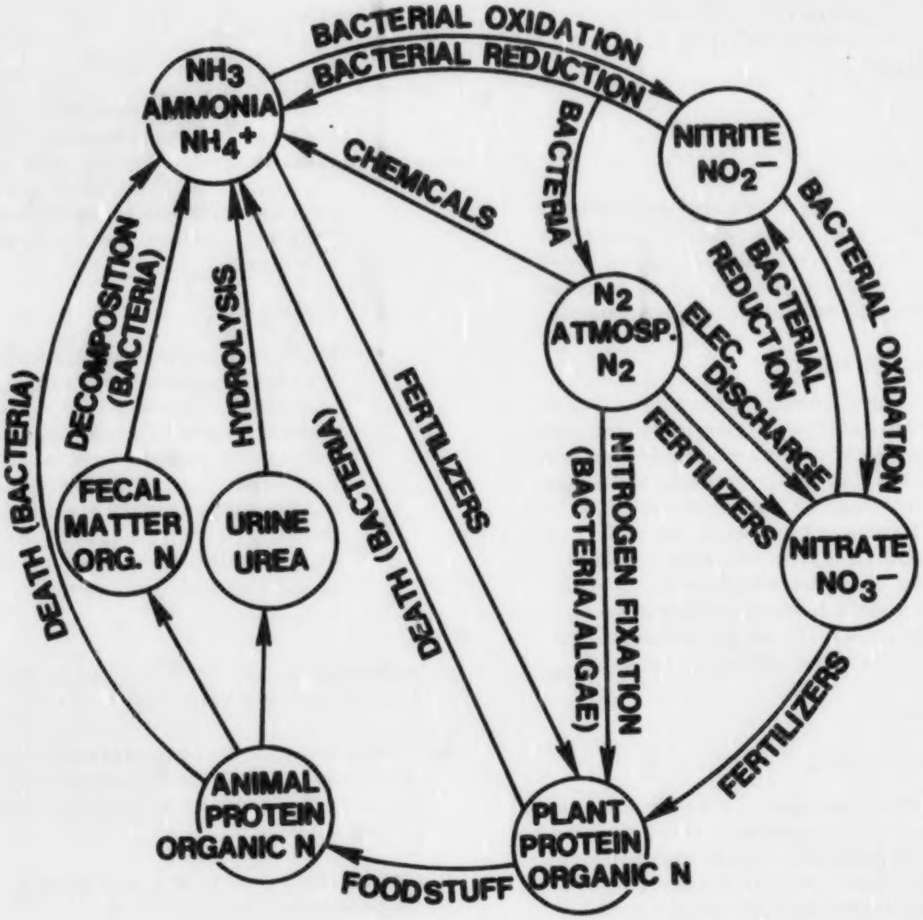
B For nitrite, cool to 4°C and analyze as soon as possible.

C Mercuric chloride is effective as a preservative but its use is discouraged because:

1 The Hg ion interferes with some of the nitrogen tests.

2 The Hg presents a disposal problem.

D Even when "preserved", conversion from one nitrogen form to another may occur. Samples should be analyzed as soon as possible.


NH3
AMMONIA
NH4+
BACTERIAL OXIDATION
BACTERIAL REDUCTION
NITRITE
NO2−
BACTERIA
CHEMICALS
BACTERIAL OXIDATION
BACTERIAL REDUCTION
N2
ATMOSP.
N2
ELEC. DISCHARGE
FERTILIZERS
NITRATE
NO3−
DECOMPOSITION (BACTERIA)
HYDROLYSIS
DEATH (BACTERIA)
DEATH (BACTERIA)
FERTILIZERS
NITROGEN FIXATION (BACTERIA/ALGAE)
FECAL MATTER ORG. N
URINE UREA
FERTILIZERS
ANIMAL PROTEIN ORGANIC N
PLANT PROTEIN ORGANIC N
FOODSTUFF

THE NITROGEN CYCLE

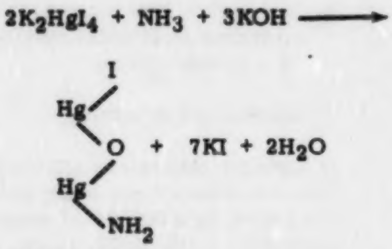
Figure 1

III DETERMINATION OF AMMONIA

A Nesslerization

1 Reaction

Nessler's reagent, a strong alkaline solution of potassium mercuric iodide, combines with NH_3 in alkaline solution to form a yellowish brown colloidal dispersion.



$$2K_2HgI_4 + NH_3 + 3KOH \longrightarrow \mathrm{I{-}Hg{-}O{-}Hg{-}NH_2} + 7KI + 2H_2O$$

Yellow-Brown

The intensity of the color follows the Beer-Lambert Law and exhibits maximum absorption at 425 nm.

2 Interferences

a Nessler's reagent forms a precipitate with some ions (e.g., Ca^{++}, Mg^{++}, Fe^{+++}, and $S^{=}$). These ions can be eliminated in a pretreatment flocculation step with zinc sulfate and alkali. Also, EDTA or Rochelle salt solution prevents precipitation with Ca^{++} or Mg^{++}.

b Residual chlorine indicates ammonia may be present in the form of chloramines. The addition of sodium thiosulfate will convert these chloramines to ammonia.

c Certain organics may produce an off color with Nessler's reagent. If these compounds are not steam distillable, the interference may be eliminated in the distillation method.

d If the turbidity and natural color of the sample cannot be eliminated with flocculation, it is then necessary to use the following distillation method.

B Distillation$^{(8)}$

1 Reaction

a The sample is distilled in the presence of a borate buffer at pH 9.5

$$NH_4 + \xrightarrow{\Delta} NH_3 + H^+$$

$$H^+ + \underset{\text{Buffer}}{Na_2B_4O_7} \longrightarrow \text{pH 9.5 maintained}$$

b The ammonia in the distillate is then measured by either of two techniques.

1) Nesslerization is used for samples containing less than 1 mg/l of ammonia nitrogen.

2) Absorption of NH_3 by boric acid and back titration with a standard strong acid is more suitable for samples containing more than 1 mg NH_3 N/l.

$$NH_3 + HBO_2 \longrightarrow NH_4^+ + BO_2^-$$

$$BO_2^- + H^+ \xrightarrow[+]{\text{Methyl Red}} HBO_2$$

Methylene Blue

pH 7.8 - 8.0 Green

pH 6.8 - 7.0 Purple

2 Interferences

a Cynate may hydrolyze, even at pH 9.5.

b Volatile organics may come over in the distillate, causing an off-color for Nesslerization. Aliphatic and aromatic amines cause positive interference by reacting in the acid titration. Some of these can be boiled off at pH 2 to 3 prior to distillation.

c Residual chlorine must be removed by pretreatment with sodium thiosulfate.

d If a mercury salt was used for preservation, the mercury ion must be complexed with sodium thiosulfate (0.2 g) prior to distillation.

3 Precision and Accuracy(8)

Twenty-four analysts in sixteen laboratories analyzed natural water samples containing the following amounts of ammonia nitrogen: 0.21, 0.26, 1.71, and 1.92 mg NH_3-N/liter.

a Precision

The standard deviation was: 0.122, 0.070, 0.244, and 0.279 mg NH_3-N/liter, respectively.

b Accuracy

The bias was: -0.01, -0,05, +0.01, and -0.04 mg NH_3-N/liter, respectively.

C Selective Ion Electrode(8)

1 Principle

A hydrophobic, gas-permeable membrane is used to separate the sample solution from an ammonium chloride internal solution. The ammonia in the sample diffuses through the membrane and alters the pH of the internal solution, which is sensed by a pH electrode. The constant level of chloride in the internal solution is sensed by a chloride selective ion electrode which acts as the reference electrode.

2 Interferences

a Volatile amines are a positive interference.

b Mercury forms a complex with ammonia so it should not be used as a preservative.

3 Precision and Accuracy

In a single laboratory (EPA) four surface water samples were analyzed containing the following amounts of ammonia nitrogen: 1.00, 0.77, 0.19, and 0.13 mg NH_3-N/liter.

a Precision

The standard deviations were 0.038, 0.017, 0.007, and 0.003 mg NH_3-N/liter, respectively.

b Accuracy

The % recovery on the 0.19 and 0.13 concentrations was 96% and 91% respectively.

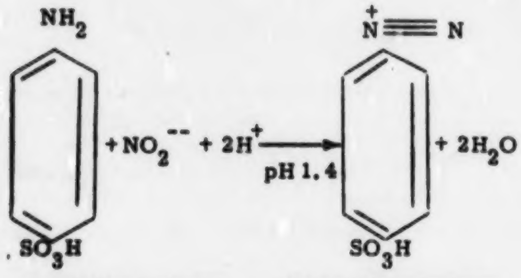
IV DETERMINATION OF NITRITE

A Diazotization(8)

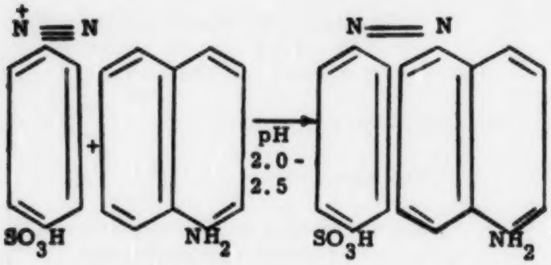
1 Reaction

a Under acid conditions, nitrite ions react with sulfanilic acid to form a diazo compound.

b The diazo compound then couples with α-naphthylamine to form an intense red azo dye which exhibits maximum absorption at 540 nm.


NH2
+NO2-- + 2H+
pH 1.4
N≡N
+ 2H2O
SO3H
SO3H

SULFANILIC ACID DIAZO COMPOUND


N≡N
+
pH 2.0 - 2.5
N═N
SO3H
NH2
SO3H
NH2

2 Interferences

a There are very few known interferences at concentrations less than 1000 times that of nitrite.

b High alkalinity (greater than 600 mg/liter) will give low results due to a shift in pH.

3 Precision

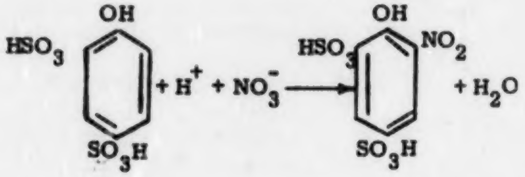
On a synthetic sample containing 0.25 mg nitrite nitrogen/l, the ARS Water Minerals Study (1961) reported 125 results with a standard deviation of ±0.029 mg/l.

V DETERMINATION OF NITRATE

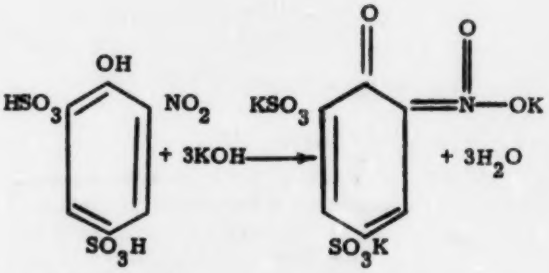
A Phenoldisulfonic Acid[(6)]

1 Reaction

a Phenoldisulfonic acid reacts with nitrate to produce a nitro derivative.


OH
HSO3
+ H+ + NO3-
SO3H
OH
HSO3
NO2
+ H2O
SO3H

b In alkaline solution the nitro derivative rearranges to form a yellow-colored compound which exhibits maximum absorption at 410 nm.


OH
HSO3
NO2
+ 3KOH
SO3H
O
O
KSO3
N—OK
+ 3H2O
SO3K

COLORLESS YELLOW

2 Interferences

a Chloride ion under the acid conditions of the test introduces negative interference.

$$6Cl^- + 2NO_3^- + 8H^+ \longrightarrow 3Cl_2\uparrow + 2NO\uparrow + 4H_2O$$

Silver sulfate can be used to precipitate Cl^-, but due to incomplete precipitation of Ag^+, an offcolor or turbidity is produced when the final color is developed. (Note: This difficulty can be overcome by using NH_4OH as the alkali.)

b Nitrites in concentrations greater than 0.2 mg N/l introduce positive interference. However, in most waters, the concentration of nitrite is insignificant compared to nitrate.

c Color and turbidity may be removed by using $Al(OH)_3$ suspension or by flocculation with $ZnSO_4$ and alkali.

3 Precision and accuracy

On a synthetic sample containing 1.0 mg nitrate N/l, and 200 mg Cl^-/l, the ARS Water Nutrients Study (1966) reported 46 results with a standard deviation of ±0.399 mg/l and a mean error of -0.31 mg/l.

B Brucine Sulfate(6, 7, 8)

1 Reaction

Brucine, a strychnine compound reacts with nitrate to form a yellow compound which exhibits maximum absorption at 410 nm. The reaction according to the procedure as outlined in Standard Methods does not follow Beer's Law. However, a recent modification by Jenkins and Medsker (2) has been developed. Conditions are controlled in the reaction so that Beer's Law is followed and concentrations below 1 mg nitrate N/l can be determined.

2 Interferences

a Nitrite may react the same as nitrate but can be eliminated by the addition of sulfanilic acid to the brucine reagent.

b Organic nitrogen compounds may hydrolyze and give positive interference at low (less than 1 mg/l) nitrate nitrogen concentrations.

c Residual chlorine may be eliminated by the addition of sodium arsenite.

3 Precision and Accuracy(8)

a Twenty-seven analysts in 15 laboratories analyzed in natural water samples containing the following increments of inorganic nitrate: 0.16, 0.19, 1.08 and 1.24 mg N/liter.

b Precision results as standard deviation were 0.092, 0.083, 0.245, and 0.214 mg N/liter respectively.

c Accuracy expressed as bias was -0.01, +0.02, +0.04 and +0.04 mg N/liter, respectively.

C Cadmium Reduction(6, 8)

1 Reaction

A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (that originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form an intensely red azo dye which is measured spectrophotometrically.

To obtain the value for only nitrate, more of the filtered sample is tested using the same colorimetric reaction but without passing it through the reduction column. The resulting value represents the nitrite originally present in the sample. Subtracting this nitrite value for the non-reduced sample from the nitrate + nitrite value for the reduced sample gives the value for nitrate originally present in the sample.

2 Interferences

a Build-up of suspended matter in the reduction column will restrict sample flow. Filtration or flocculation with zinc sulfate should remove turbidity.

b High concentrations of iron, copper or other metals may interfere. EDTA may be used to complex these.

c Large concentrations of oil and grease in a sample can coat the surface of the cadmium. Pre-extracting the sample with an organic solvent removes oil and grease.

3 Precision and accuracy(6) ·

In 11 laboratories, three samples were analyzed containing the following amounts of nitrate nitrogen· 0.05, 0.5, and 5 mg NO_3-N/liter.

a Precision

The relative standard deviation was 96.4%, 25.6%, and 9.2%, respectively.

b Accuracy

The relative error was 47.3%, 6.4%, and 1.0%, respectively.

4 Automated cadmium reduction

The EPA Methods Manual(8) contains details for the automated procedure.

D Hydrazine Reduction

A method using hydrazine to reduce nitrate to nitrite followed by subsequent measurement of nitrite by diazotization was reported by Fishman, et al. (1)

The means to determine nitrate is the same as above in the Cadmium Reduction Method. Subtraction of nitrite (determined from non-reduced sample) from the total nitrite (reduced nitrate + original nitrite) will give the original nitrate nitrogen concentration.

The procedure was adapted to the Auto Analyzer by Kamphake, et al. (3) and can be found in detail in the 1971 edition of the EPA Methods Manual. (8)

REFERENCES

1 Fishman, Marvin J., Skougstad, Marvin W., and Scarbio, George, Jr Diazotization Method for Nitrate and Nitrite. JAWWA 56:633-638 May, 1960

2 Jenkins, David and Medsker, Lloyd L. Brucine Method for Determination of Nitrate in Ocean, Estuarine and Fresh Waters. Anal. Chem. 36:610-612. March, 1964.

3 Kamphake, L. J., Hannah, S. and Cohen, J. Automated Analysis for Nitrate by Hydrazine Reduction. Water Research, 1, 205. 1967.

4 Lishka, R. J., Lederer, L. A., and McFarren, E. F. Water Nutrients No. 1, Analytical Reference Service 1966.

5 Sawyer, Clair N. Chemistry for Sanitary Engineers. McGraw-Hill Book Co., New York. 1960.

6 Standard Methods for the Examination of Water and Waste Water. APHA. AWWA. WPCF. 13th Ed. 1971.

7 ASTM Book of Standards, Part 23, 1970.

8 Methods for Chemical Analysis of Water & Wastes, EPA-MDQARL, Cincinnati, Ohio 45268. 1974.

This outline was prepared by B. A. Punghorst, former Chemist, and C. R. Feldmann, Chemist, and revised by A. E. Donahue, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Ammonia, Chemical Analysis, Nitrates, Nitrites, Nitrogen, Nitrogen Compounds, Nitrogen Cycle, Nutrients, Water Analysis, Water Pollution Sources

TOTAL CARBON ANALYSIS

I INTRODUCTION

A History of Carbon Analyses

In the wake of a rapid population growth, and the increasing heavy use of our natural waterways, the nation, and indeed the world, is presented with the acute problem of increased pollutional loads on streams, rivers and other receiving bodies. This has resulted in a growing awareness of the need to prevent the pollution of streams, rivers, lakes and even the oceans. Along with this awareness has developed a desire for a more rapid and precise method of detecting and measuring pollution due to organic materials.

B The Methods

In the past, two general approaches have been used in evaluating the degree of organic water pollution.

1 The determination of the amount of oxygen or other oxidants required to react with organic impurities.

2 The determination of the amount of total carbon present in these impurities.

C Oxygen Demand Analyses

The first approach is represented by conventional laboratory tests for determining Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD). One of the principal disadvantages of these tests is that they are limited primarily to historical significance, that is, they tell what a treatment plant had been doing, since they require anywhere from two hours to five days to complete. Since up to now no faster method has been available, traditional BOD and COD determinations have become accepted standards of measure in water pollution control work even though they are essentially ineffective for process control purposes.

Until the introduction of the Carbonaceous Analyzer, all methods taking the second approach, the total carbon method of evaluating water quality, also proved too slow.

II THE ANALYSIS OF CARBON

A Pollution Indicator

Now the carbonaceous analyzer provides a means to determine the total carbon content of a dilute water sample in approximately two minutes. With proper sample preparation to remove inorganic carbonates, the instrument determines the total organic carbon content in the sample.

B Relationship of Carbon Analysis to BOD and COD

This quantity varies with the structure from 27 percent for oxalic acid through 40 percent for glucose to 75 percent for methane. The ratio of COD to mg carbon also varies widely from 0.67 for oxalic acid through 2.67 for glucose to 5.33 for methane. Representative secondary sewage effluents have given a ratio of COD to carbon content of between 2.5 and 3.5 with the general average being 3.0.

The BOD, COD and carbon contents of these and some other representative compounds are summarized in the following table.

Sample	5-Day BOD-mg/mg	COD-mg/mg	% Carbon
Stearic Acid - $C_{18}H_{36}O_2$	.786	2.91	76
Glucose - $C_6H_{12}O_6$	.73	1.07	40
Oxalic Acid - $C_2H_2O_4$	.14	.18	27
Benzoic Acid - $C_7H_6O_2$	1.38	1.97	69
Phenol - C_6H_6O	.05 to 2.1 depending upon concentration	2.36	77
Potassium Acid Phthalate $KHC_8H_4O_4$	.95	1.15	47
Salicylic Acid - $C_7H_6O_3$	1.25	1.60	61
Secondary Effluent, Clarified	13*	75*	21*
" " "	23*	67*	12*
" " "	4*	36*	7*

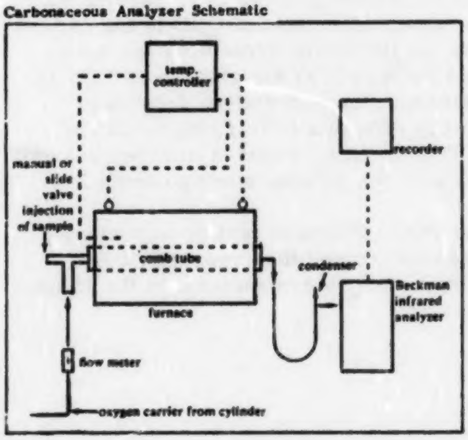
*In units of mg/l

III THE CARBON ANALYZER

A Principle of Operation

Basically the carbonaceous analyzer consists of three sections - a sampling and oxidizing system, a Beckman Model 315 Infrared Analyzer, and a strip-chart recorder.

Carbonaceous Analyzer Schematic


temp. controller
recorder
manual or slide valve injection of sample
comb tube
condenser
Beckman infrared analyzer
furnace
flow meter
oxygen carrier from cylinder

A micro sample (20-40 µl) of the water to be analyzed is injected into a catalytic combustion tube which is enclosed by an electric furnace thermostated at 950°C. The water is vaporized and the carbonaceous material is oxidized to carbon dioxide (CO_2) and steam in a carrier stream of pure oxygen. The oxygen flow carries the steam and CO_2 out of the furnace where the steam is condensed and the condensate removed. The CO_2, oxygen and remaining water vapor enter an infrared analyzer sensitized to provide a measure of CO_2. The output of the infrared analyzer is recorded on a strip chart, after which, the curve produced can be evaluated by comparing peak height with a calibration curve based upon standard solutions. Results are obtained directly in milligrams of carbon per liter.

B Application

Results(1) show that the method is applicable for most, if not all, water-soluble organic compounds -- including those that contain sulfur, nitrogen, and volatiles.

Nonvolatile organic substances can be differentiated from volatiles, such as carbon dioxide or light hydrocarbons by determination of carbon both before and after the sample solution has been blown with an inert gas.

C Sample Preparation

The Carbonaceous Analyzer is often referred to as a total carbon analyzer because it provides a measure of all the carbonaceous material in a sample, both organic and inorganic. However, if a measure of organic carbon alone is desired, the inorganic carbon content of the sample can be removed during sample preparation.

1 Removal of inorganic carbon

The simplest procedure for removing inorganic carbon from the sample is one of acidifying and blowing. A few drops of HCl per 100 ml of sample will normally reduce pH to 2 or less, releasing all the inorganic carbon as CO_2. Five minutes of blowing with a gas free of CO_2 sweeps out the CO_2 formed by the inorganic carbon. Only the organic carbon remains in the sample and may be analyzed without the inorganic interference.

2 Volatile carbonaceous material

Volatile carbonaceous material that may be lost by blowing is accounted for by using a dual channel carbon analyzer. Beckman's new analyzer has the previously detailed high temperature (950°C) furnace plus a low temperature (150°C) one. Using quartz chips wetted with phosphoric acid, the low temperature channel senses only the CO_2 (freed by the acid) in the original sample. The remaining organics and water are retained in the condenser connected to this low temperature furnace. None of the organics are oxidized by the 150°C furnace.

By injecting a sample into the low temperature furnace, a peak representing the inorganic carbon is obtained on the strip chart. Injecting a nonacidified sample into the high temperature furnace yields a peak representing the total carbon. The difference between the values determined for the two peaks is the total organic carbon.

3 Dilute samples

If the sample is dilute (less than 100 mg/liter carbon) and is a true solution (no suspended particles) no further preparation is required.

4 Samples containing solids

If the sample contains solids and/or fibers which are to be included in the determination, these must be reduced in size so that they will be able to pass through the needle which has an opening of 170 microns (needles having larger openings may be obtained if necessary). In most cases, mixing the sample in a Waring Blender will reduce the particle size sufficiently for sampling.

IV PROCEDURE FOR ANALYSIS

A Interferences

Water vapor resulting from vaporization of the sample, causes a slight interference in the method. Most of the water is trapped out by the air condenser positioned immediately after the combustion furnace. However, a portion of the water vapor passes through the system into the infrared detector and appears on the strip chart as carbon. The water blank also appears on the standard calibration curve, and is therefore removed from the final calculation. In tests of solutions containing the following anions: NO_3^-, Cl^-, SO^{-2}, PO_4^{-3}, no interference was encountered with concentrations up to one percent.

B Precision and Accuracy

The recovery of carbon from standard solutions is 98.5 - 100.0 percent. The minimum detectable concentration using the prescribed operating instructions is 1 mg/1 carbon. Generally, the data are reproducible to ± 1 mg/1 with a standard deviation of 0.7 mg/1 at the 100 mg/1 level.

V APPLICATIONS

Several of the many research and industrial applications of the Carbonaceous Analyzer are listed below:

A Determine the efficiency of various wastewater renovation processes, both in the laboratory and in the field.

B Compare a plant's waste outlet with its water inlet to determine the degree of contamination contributed.

C Monitoring a waste stream to check for product loss.

D Follow the rate of utilization of organic nutrients by micro-organisms.

E To detect organic impurities in inorganic compounds.

VI ADVANTAGES OF CARBON ANALYZER

A Speed

The Carbonaceous Analyzer's most important advantage is its speed of analysis. One analysis can be performed in 2-3 minutes. This speed of analysis brings about another advantage, economy of operation. Working with dilute samples, one man can run ten to twenty carbon determinations per hour. This is probably more than the number of COD or BOD tests that can even be started, much less completed, in the same period of time.

B Total Carbon

Another advantage is that the measure of carbon is a total one. The oxidizing system of the analyzer brings about complete oxidation of any form of carbon. No compound has been found to which the method is inapplicable.

VII CONCLUSIONS

The Carbonaceous Analyzer provides a rapid and precise measurement of organic carbon in both liquid and air samples. It should be found useful for many research and industrial applications, a few of which have been mentioned.

Because of its rapidity it may be found more useful than the more time-consuming BOD and COD measurements for monitoring industrial waste streams or waste treatment processes.

REFERENCES

1 Van Hall, C. E., Safranko, John and Stenger, V. A. Anal. Chem. 35, 315-9. 1963.

2 Van Hall, C. E., and Stenger, V. A. Draft of Final Report - Phase I - Contract PH 86-63-94, Analytical Research Toward Application of the Dow Total Carbon Determination Apparatus to the Measurement of Water Pollution.

3 Van Hall, C. E., Stenger, V. A. Beckman Reprint - R6215. Taken from Paper Presented at the Symposium on Water Renovation, Sponsored by the Division of Water and Waste Chemistry. ACS in Cincinnati. Jan. 14-16, 1963.

This outline was prepared by Robert T. Williams, Chief, and revised by Charles J. Moench, Jr., Waste Identification and Analysis Section, MERL, USEPA, Cincinnati, Ohio 45268.

Descriptors: Biochemical Oxygen Demand, Carbon, Chemical Analysis, Chemical Oxygen Demand, Organic Matter, Organic Wastes, Water Analysis, Instrumentation, Nutrients

DETERMINATION OF CALCIUM AND MAGNESIUM HARDNESS

I INTRODUCTION

A Definition of Hardness

USPHS - "In natural waters, hardness is a characteristic of water which represents the total concentration of just the calcium and magnesium ions expressed as calcium carbonate. If present in significant amounts, other hardness-producing metallic ions should be included."

B Other Definitions in Use

1 Some confusion exists in understanding the concept of hardness as a result of several definitions presently used.

2 Soap hardness definition includes hydrogen ion because it has the capacity to precipitate soap. Present definition excludes hydrogen ion because it is not considered metallic.

3 Other agencies define hardness as "the property attributable to presence of alkaline-earths".

4 USPHS definition is best in relation to objections of hardness in water.

II CAUSES OF HARDNESS IN WATERS OF VARIOUS REGIONS OF THE U. S.

A Hardness will vary throughout the country depending on:

1 Leaching action of water traversing over and through various types of geological formations.

2 Discharge of industrial and domestic wastes to water courses.

3 Uses of water which result in change in hardness, such as irrigation and water softening process.

B Objections to Hardness

1 Soap-destroying properties

2 Scale formation

C Removal and Control

Hardness may be removed and controlled through the use of various softening operations such as zeolite, lime-soda, and hot phosphate processes. It can also be removed by simple distillation or complex formation with surface active agents (detergents).

III DETERMINATION OF HARDNESS

A Two Methods in Use(6)

1 Compleximetric method (EDTA)

2 Calculation - by the use of appropriate factors, the hardness due to ions other than calcium and magnesium may be converted to an equivalent calcium carbonate hardness.

B Compleximetric Method

1 Principle of determination

Ethylenediaminetetra acetic acid (EDTA) is a sparingly soluble amino polycarboxylic acid which forms slightly ionized and very stable colorless complexes with the alkaline-earth metals.

2 Interferences: iron, manganese, nickel, and zinc.

3 Procedure: Time and pH considerations.

4 Calculations of total hardness assuming a known volume of titrant of EDTA.

5 Precision and accuracy.

C Determination of Calcium Hardness

1 Principle of determinations

Murexide indicator forms a salmon-colored complex with calcium whose ionization constant is of a higher value than that of the Ca EDTA complex.

2 Interferences: Heavy metals and Sr.

3 Procedure: Time of tritation and proper lighting conditions are critical factors.

4 Calculation of Ca hardness.

5 Precision and accuracy.

D Determination of Magnesium Hardness

1 Calculation by difference method most commonly used.

2 Equivalent of Ca hardness is subtracted from total hardness equivalents, the difference attributable to magnesium equivalents.

3 Other methods such as pyrophosphate method, where calculation by difference method cannot be used.

IV ANALYTICAL QUALITY CONTROL METHODS

A The Environmental Protection Agency, Analytical Quality Control Laboratory has published a manual titled Methods for Chemical Analysis of Water and Wastes, 1974.

B The procedure for the determination of hardness, as discussed in this manual, was excerpted from the 13th ed. of Standard Methods(4) and part 23 of ASTM.(5)

REFERENCES

1 Barnard, A.J. Jr., Broad, W.C. and Flaschka, H. The EDTA Titration. J.T. Baker Company. 1957.

2 U.S. Public Health Service. Drinking Water Standards. USPHS Report, Volume 61, No. 11, 1946.

3 Rainwater, R.H. and Thatcher, L.L. Methods for the Collection and Analysis of Water Samples. U.S. Geological Survey Water Supply, Paper 1454. 1960.

4 Standard Methods for the Examination of Water and Wastewater, 13th ed. APHA, AWWA, WPCF, Method 122B, 1971.

5 ASTM Standards, Part 23, Method D1126-67, 1973.

6 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL, Cincinnati, OH, 1974.

This outline was prepared by B. V. Salotto, Research Chemist, Waste Identification and Analysis Section, MERL, EPA, and revised by C. R. Feldmann, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, OH 45268.

Descriptors: Calcium, Chemical Analysis, Hardness, Magnesium, Water Analysis

SOLIDS RELATIONS IN POLLUTED WATER

I MPN, oxygen demand, and solids have been major water pollution control criteria for many years. This discussion is concerned primarily with solids and their interrelations with oxygen demand.

A Engineered treatment or surface water self-purification depends upon:

1 The conversion of soluble or colloidal contaminants into agglomerated masses that may be separated from the water.

2 Oxidation of putrescible components into stable degradation products.

3 Item 1 is the major concern in most treatment systems because item 2 requires a greater investment in time, manpower and capital costs.

B Stress on oxygen demand removal frequently results in an unduly small amount of attention to the contribution of solids in the oxygen demand picture.

1 Oxygen demand formulations generally are specified to be applicable in the absence of significant deposition.

2 Increasing impoundment, tidal estuaries, and incomplete solids removal, generally ensure that solids deposition will be significant.

3 The BOD test stresses the fraction of oxygen demand that is exerted relatively rapidly. It includes only the fraction of unstable material that is exerted under test conditions - usually short term under aerobic conditions.

4 Contributions of a bed load of solids to oxygen demand frequently are incompletely recognized because they have a more local effect, are difficult to measure, tend to move, and are incompletely understood. The onset of an aerobic action in deposited solids increases hydrolysis rate. High molecular weight cell mass splits into small molecules that are soluble and more available for high rate oxidation upon feedback into oxygen containing water.

II A given wastewater may have several forms of solids in changeable proportions with time and conditions.

A Solids may be classified among the charted forms according to biological chemical or physical properties. It is not possible to precisely classify a given material into any one form because they usually are mixtures that may include or be converted into other forms.

B Interrelationships are indicated by diagram in Figure 1. Some changes occur more readily than others.

1 Settleable solids generally consist of a mixture of organic, inorganic, entrained dissolved or colloidal solids with living and dead organisms.

a They may be hydrolyzed into smaller sizes to aquire colloidal or dissolved characteristics.

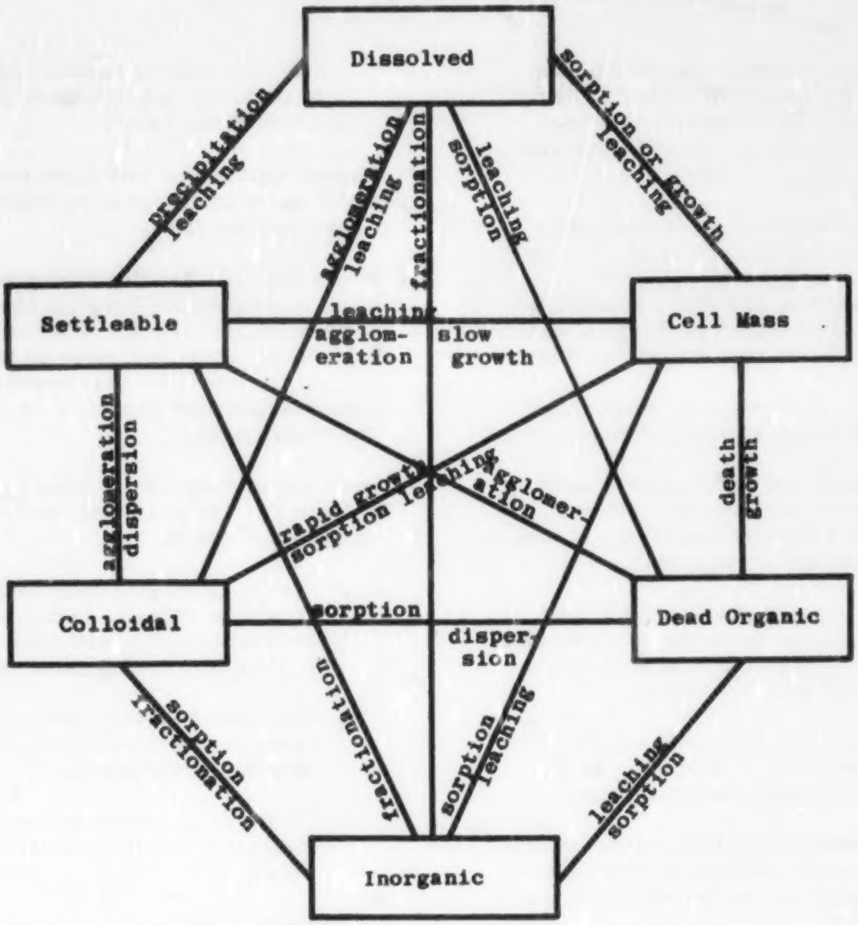
b They may be converted into a larger fraction of living material or vice versa.

2 Colloidal solids also are likely to be a mixture of organic and inorganic materials, living or dead containing associated dissolved materials.

a They are likely to agglomerate to form settleable masses with time or changing conditions.

b Chemical reactions may solubilize the colloidal masses for recombination into other forms.

3 Dissolved solids are most readily available of all forms for biological, chemical or physical conversion.


Dissolved
Settleable
Cell Mass
Colloidal
Dead Organic
Inorganic
precipitation
leaching
agglomeration
leaching
fractionation
leaching
sorption
sorption or growth
leaching
leaching
agglom-
eration
slow
growth
agglomeration
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death
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fractionation
sorption
leaching
leaching
sorption

Figure 1. SOLIDS INTERRELATIONSHIPS IN WATER

a They may be assimilated into cell mass to become colloidal during a state of rapid growth or form settleable masses during slow growth. Surface phenomena are likely to encourage inclusion of other forms of solids with cell mass.

C Stabilization occurs with each conversion because energy is expended with each change.

1 Biological changes involve oxidation to obtain enough energy to synthesize cells.

a Products consist of cell mass and degradation products.

b Oxidation tends toward production of CO_2, H_2O, NO_3^-, SO_4^{-2}etc. CO_2 has limited water solubility and partially leaves the aqueous environment. More soluble constituents tend to remain with it for recycle.

c Biological residues tend to recycle. Eventually the mass consists of relatively inert and largely insoluble residues. These make up the bed load that may decompose at a rate of less than 1% per day, but accumulates in mass until it becomes dominant in water pollution control activities.

III Treatment is an engineered operation designed to utilize events in surface water self-purification in a smaller package in terms of space and time. Effective treatment presupposes oxidation either in the plant or in facilities to minimize feedback of solids components to the aqueous environment.

A Primary treatment consists of a separation of floatable or settleable solids and removal from the used water.

1 Prompt removal is essential to minimize return of solubilized or leached materials from the sludge mass.

2 Sludge and scum are highly putrescible and difficult to drain.

3 Subsequent treatment prior to disposal serves to enhance drainability, reduce solids or volume for burial or burning.

B Secondary treatment generally involves some form of aerobic biological activity to oxidize part of the colloidal and dissolved contaminants and convert most of the remainder into a settleable sludge.

1 Aerobic systems favor assimilation of nutrients into cell mass at the expense of part of the available energy represented by oxidation to products such as CO_2 and water.

2 Cell mass and intermediate degradation products are only partially stabilized and tend to recycle with death and decay.

3 Rapid growth of cells tends to produce sludges that are highly hydrated and difficult to concentrate.

4 A compromise must be reached to produce a favorable balance among separation of solids and feedback of lysed materials.

a Cell growth continues as long as energy and nutrients permit.

b Under limiting nutrient conditions the population may show a relatively low rate overall dieoff but variety is changing.

c Species most favored by the new conditions tend to grow while others die, lyse and release part of their stored nutrients for subsequent use.

C Anaerobic digestion of solids separated during treatment is one process used to increase solids stability and drainability while reducing total volume or mass for disposal.

1 Growth of cell mass is relatively slow under anaerobic conditions while hydrolytic cleavage is relatively large in comparison to that during aerobic metabolism.

a Feedback to the aqueous environment represents a significant fraction of the input in the form of ammonia, colloidal solids, low molecular weight acids, and other products.

b Mass of the sludge is reduced by the fraction of methane, carbon dioxide, and other gases produced in process.

c Remaining solids tend to be more concentrated, are lower in putrescibility and give up their water more readily.

2 The liquid fraction of the products remaining after anaerobic digestion contain nutrients, oxygen demand, solids, and malodorous constituents that are objectionable in surface waters if released without further aerobic stabilization.

3 Digester liquids are much more concentrated than raw sewage and nutritionally unfavorable, hence they are difficult to treat and tend to shock aerobic treatment processes.

D Suitable disposal of solids resulting from treatment operations takes various forms of which the most desirable is to oxidize it completely to gaseous products of oxidation or inert ash. The objective is to limit feedback into the aqueous environment and delay the charted interchanges.

1 Deposition of sludge as a cover on crop land is effective providing surface drainage is properly designed to limit washoff.

2 Burial in areas above the flood plain provides a suitable disposal.

3 Incineration under controlled conditions to produce complete burning of organics is effective but requires close control.

a Incineration of digested sludge is feasible but the digestion process results in removal of part of the heat content of the sludge as methane carbon dioxide and water. Auxilary heat may be required for water evaporation if solids concentration is below 25%.

b Raw sludge incineration relieves the auxilary heat problem but storage may lead to odor problems. The raw sludge may require chemical treatment to enhance dewatering, secondary sludges are more difficult to dewater and may require centrifugation, floatation, or other treatment to concentrate sludge for heat balance purposes.

c Incineration requires close control of complex equipment to maintain acceptable environmental control. Usually, after burners and off gas scrubbers are necessary to avoid air pollution.

IV ANALYTICAL PROCEDURES

A The Analytical Quality Control Laboratory, Office of Water Programs, Environmental Protection Agency has published a manual titled, Methods for Chemical Analysis of Water and Wastes, 1974.

B In this manual solids are classified into four groups:

1 Residue, Total Filterable
2 Residue, Total Non-Filterable
3 Residue, Total
4 Residue, Volatile

C The procedure for the determination of 1, 2, and 3 above is given in the EPA Manual.

For the Volatile Residue procedure, Standard Methods for the Examination of Water and Wastewater, 13th Ed., page 536, Method 224B(1971) is the reference cited by the EPA Manual.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Anaerobic Digestion, Oxygen, Oxygen Demand, Sludge, Sludge Disposal, Sludge Treatment, Solids, Waste Water Treatment, Water Pollution Sources

PHOSPHORUS IN THE AQUEOUS ENVIRONMENT

I Phosphorus is closely associated with water quality because of (a) its role in aquatic productivity such as algal blooms, (b) its sequestering action, which causes interference in coagulation, (c) the difficulty of removing phosphorus from water to some desirable low concentration, and (d) its characteristic of converting from one to another of many possible forms.

A Phosphorus is one of the primary nutrients such as hydrogen (H), carbon (C), nitrogen (N), sulfur (S) and phosphorus (P).

1 Phosphorus is unique among nutrients in that its oxidation does not contribute significant energy because it commonly exists in oxidized form.

2 Phosphorus is intimately involved in oxidative energy release from and synthesis of other nutrients into cell mass via:

a Transport of nutrients across membranes into cell protoplasm is likely to include phosphorylation.

b The release of energy for metabolic purposes is likely to include a triphosphate exchange mechanism.

B Most natural waters contain relatively low levels of P (0.01 to 0.05 mg/l) in the soluble state during periods of significant productivity.

1 Metabolic activity tends to convert soluble P into cell mass (organic P) as a part of the protoplasm, intermediate products, or sorbed material.

2 Degradation of cell mass and incidental P compounds results in a feedback of lysed P to the water at rates governed by the type of P and the environment. Aquatic metabolic kinetics show marked influences of this feedback.

3 The concentrations of P in hydrosoils, sludges, treatment plant samples and soils may range from 10^2 to 10^6 times that in stabilized surface water. Both concentration and interfering components affect applicability of analytical techniques.

II The primary source of phosphorus in the aqueous system is of geological origin. Indirect sources are the processed mineral products for use in agriculture, household, industry or other activities.

A Agricultural fertilizer run-off is related to chemicals applied, farming practice and soil exchange capacity.

B Wastewaters primarily of domestic origin contain major amounts of P from:

1 Human, animal and plant residues

2 Surfactants (cleaning agent) discharge

3 Microbial and other cell masses

C Wastewaters primarily of industrial origin contain P related to:

1 Corrosion control

2 Scale control additives

3 Surfactants or dispersants

4 Chemical processing of materials including P

5 Liquors from clean-up operations of dusts, fumes, stack gases, or other discharges

III Phosphorus terminology is commonly confused because of the interrelations among biological, chemical, engineering, physical, and analytical factors.

A Biologically, phosphorus may be available as a nutrient, synthesized into living mass, stored in living or dead cells, agglomerates, or mineral complexes, or converted to degraded materials.

B Chemically, P exists in several mineral and organic forms that may be converted from one to another under favorable conditions. Analytical estimates are based upon physical or chemical techniques necessary to convert various forms of P into ortho phosphates which alone can be quantitated in terms of the molybdenum blue colorimetric test.

C Engineering interest in phosphorus is related to the prediction, treatment, or control of aqueous systems to favor acceptable water quality objectives. Phosphorus removal is associated with solids removal.

D Solubility and temperature are major physical factors in phosphorus behavior. Soluble P is much more available than insoluble P for chemical or biological transformations and the rate of conversion from one to another is strongly influenced by temperature.

E Table 1 includes a classification of the four main types of chemical P and some of the relationships controlling solubility of each group. It is apparent that no clear-cut separation can be made on a solubility basis as molecular weight, substituent and other factors affect solubility.

F Table 2 includes a scheme of analytical differentiation of various forms of P based upon:

1 The technique required to convert an unknown variety of phosphorus into ortho P which is the only one quantitated by the colorimetric test.

2 Solubility characteristics of the sample P or more precisely the means required to clarify the sample.

a Any clarification method is subject to incomplete separation. Therefore, it is essential to specify the method used to interpret the yield factor of the separation technique. The degree of separation of solubles and insolubles will be significantly different for:

1 Membrane filter separation (0.5 micron pore size)

2 Centrifugation (at some specified rpm and time)

3 Paper filtration (specify paper identification)

4 Subsidence (specify time and conditions)

G Analytical separations (Table 2) like those in Table 1, do not give a precise separation of the various forms of P which may be included quantitatively with ortho or poly P. Conversely some of the poly and organic P will be included with ortho P if they have been partially hydrolyzed during storage or analysis. Insolubles may likewise be included as a result of poor separation and analytical conditions.

1 The separation methods provide an operational type of definition adequate in most situations if the "operation" is known. Table 2 indicates the nature of incidental P that may appear along with the type sought.

Table 1

PHOSPHORUS COMPOUNDS CLASSIFIED BY CHEMICAL AND SOLUBILITY RELATIONS

Form	Water Soluble(1)	Insoluble(1)
1. Ortho phosphates $(PO_4)^{-3}$	Combined with monovalent cations such as H^+, Na^+, K^+, NH_4^+	Combined with multi valent cations such as Ca^{+2} Al^{+3} Fe^{+3}
2. Poly phosphates $(P_2O_7),^{-4}(P_3O_{10})^{-5}(P_3O_9)^{-3}$ and others depending upon the degree of dehydration.	as in 1 above Increasing dehydration decreases solubility	(a) as in 1 above (b) multi P polyphosphates (high mol. wt.) including the "glassy" phosphates
3. Organic phosphorus R-P, R-P-R (2) (unusually varied nature)	(a) certain chemicals (b) degradation products (c) enzyme P (d) phosphorylated nutrients	(a) certain chemicals (b) cell mass, may be colloidal or agglomerated (c) soluble P sorbed by insoluble residues
4. Mineral phosphorus	(a) as in 1 above	(a) as in 1 above (b) as in 2 above (c) geological P such as phosphosilicates (d) certain mineral complexes.

(1) Used in reference to predominance under common conditions.

(2) R represents an organic radical, P represents P, PO_4^{-3}, or its derivatives.

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2 Total P in Table 2 includes liquid and separated residue P that may exist in the whole sample including silt, organic sludge, or hydrosoils. This recognizes that the feedback of soluble P from deposited or suspended material has a real effect upon the kinetics of the aqueous environment.

Table 2

PHOSPHORUS COMPOUNDS CLASSIFIED BY ANALYTICAL METHODOLOGY

Desired P Components	Technique(1)	Incidental P Included(2)
1. Ortho phosphates	No treatment on clear samples	Easily hydrolyzed (a) poly phosphates - (b) organic -P, - (c) Mineral -P, + or -
2. Polyphosphates (2)-(1) = poly P (hydrolyzable)	acid hydrolysis on clear samples, dilute (a) H_2SO_4 (b) HCl heated	(a) ortho-P + (b) organic -P + or - (c) mineral -P + or -
3. Organic phosphorus (3) - (2) + org P (hydrolyzable)	acid + oxidizing hydrolysis on whole sample. dilute (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + (NH_4)_2S_2O_8$ heated	(a) ortho P + (b) poly P + (c) mineral P + or -
4. Soluble phosphorus (preferably classified by clarification method)	clarified liquid following filtration, centrifugation or subsidence	generally includes (a) 1, 2, or 3 (b) particulates not completely separated
5. Insoluble phosphorus (residue from clarification)	Retained residues separated during clarification See (6)	(a) generally includes sorbed or complexed solubles.
6. Total phosphorus	Strong acid + oxidant digestion (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + HNO_3 + HClO_4$ (c) $H_2O_2 + Mg(NO_3)_2$ fusion	all components in 1, 2, 3, 4, 5 in the whole sample

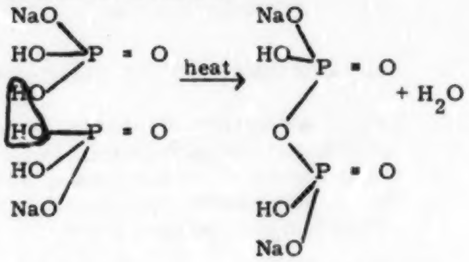
(1) Determinative step by phospho molybdate colorimetric method.

(2) Coding: + quantitative yield
- a small fraction of the amount present
+ or - depends upon the individual chemical and sample history

IV Polyphosphates are of major interest in cleaning agent formulation, as dispersants, and for corrosion control.

A They are prepared by dehydration of ortho phosphates to form products having two or more phosphate derivatives per molecule.

1 The simplest polyphosphate may be prepared as follows:



mono sodium ortho phosphate (2) disodium dihydrogen polyphosphate

2 The general form for producing polyphosphates from mono substituted orthophosphates is:

$$n\ (NaH_2PO_4) \xrightarrow[200-400^{\circ} C]{heat} (NaPO_3)_n + n\ H_2O$$

3 Di-substituted ortho phosphates or mixtures of substituted ortho phosphates lead to other polyphosphates:

$$2Na_2HPO_4 + NaH_2PO_4 \xrightarrow{heat} Na_5P_3O_{10} + 2H_2O$$

disodium hydrogen ortho phosphate + mono sodium di hydrogen ortho phosphate → pentasodium tri-phosphate + water

4 The polyphosphate series usually consist of the polyphosphate anion with a negative charge of 2 to 5. Hydrogen or metals commonly occupy these sites. The polyphosphate can be further dehydrated by heat as long as hydrogen remains. Di or trivalent cations generally produce a more insoluble polyphosphate than the same cation in the form of insoluble ortho phosphate. Insolubility increases with the number of P atoms in the polyphosphate. The "glassy" polyphosphates are a special group with limited solubility that are used to aid corrosion resistance in pipe distribution systems and similar uses.

B Polyphosphates tend to hydrolyze or "revert" to the ortho P form by addition of water. This occurs whenever polyphosphates are found in the aqueous environment.

1 The major factors affecting the rate of reversion of poly to orthophosphates include:

a) Temperature, increased T increases rate

b) pH, lower pH increases rate

c) Enzymes, hydrolase enzymes increase rate

d) Colloidal gels, increase rate

e) Complexing cations and ionic concentration increase rate

f) Concentration of the polyphosphate increases rate

2 Items a, b and c have a large effect upon reversion rate compared with other factors listed. The actual reversion rate is a combination of listed items and other conditions or characteristics.

3 The differences among ortho and ortho + polyphosphates commonly are close to experimental error of the colorimetric test in stabilized surface water samples. A significant difference generally indicates that the sample was obtained relatively close to a source of polyphosphates and was promptly analyzed. This implies that the reversion rate of polyphosphates is much higher than generally believed.

V SAMPLING AND PRESERVATION TECHNIQUES

A Sampling

1 Great care should be exercised to exclude any benthic deposits from water samples.

2 Glass containers should be acid rinsed before use.

3 Certain plastic containers may be used. Possible adsorption of low concentrations of phosphorus should be checked.

4 If a differentiation of phosphorus forms is to be made, filtration should be carried out immediately upon sample collection. A membrane filter of 0.45μ pore size is recommended for reproducible separations.

B Preservation

1 If at all possible, samples should be analyzed on the day of collection. At best, preservation measures only retard possible changes in the sample.

a Possible physical changes include solubilization, precipitation, absorption on or desorption from suspended matter.

b Possible chemical changes include reversion of poly to ortho P and decomposition of organic or mineral P.

c Possible biological changes include microbial decomposition of organic P and algal or bacterial growth forming organic P.

2 Refrigeration at 4°C is recommended if samples are to be stored up to 24 hours. This decreases hydrolysis and reaction rates and also losses due to volatility.

3 Addition of 2 ml concentrated H_2SO_4 or 40 mg $HgCl_2$/liter is recommended for longer storage periods. This chemical limits biological changes.

a $HgCl_2$ is an interference in the analytical procedure if the chloride level is low (less than 50 mg. Cl/l). See Part VI B 3 below.

VI THE EPA ANALYTICAL PROCEDURE(6)

A This is a colorimetric determination, specific for orthophosphate. Depending on the nature of the sample and on the type of data sought, the procedure involves two general operations:

1 Conversion of phosphorus forms to soluble orthophosphate (See Fig. 1):

a sulfuric acid-hydrolysis for polyphosphates, and some organic P compounds,

b persulfate digestion for organic P compounds.

2 The color determination involves reacting dilute solutions of phosphorus with ammonium molybdate and potassium antimonyl tartrate in an acid medium to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the orthophosphate concentration.

Color absorbance is measured at 880 nm or 650 nm and a concentration value obtained using a standard curve.

Reagent preparation and the detailed procedure can be found in the EPA manual.

The methods described there are usable in the 0.01 to 0.5 mg/liter phosphorus range.

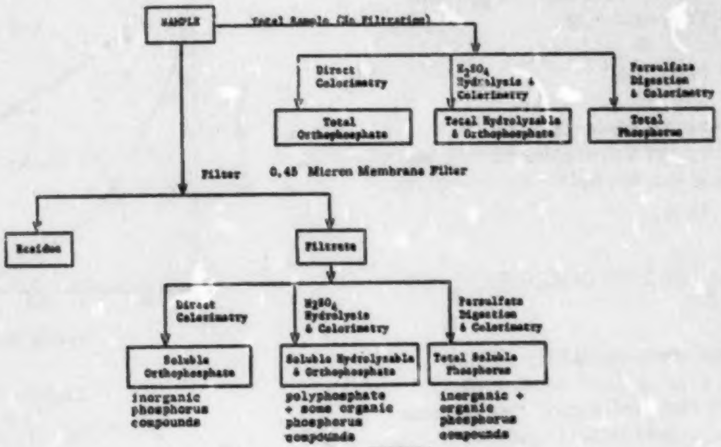

SAMPLE
Total Sample (No Filtration)
Direct Colorimetry
H_2SO_4 Hydrolysis & Colorimetry
Persulfate Digestion & Colorimetry
Total Orthophosphate
Total Hydrolyzable & Orthophosphate
Total Phosphorus
Filter
0.45 Micron Membrane Filter
Residue
Filtrate
Direct Colorimetry
H_2SO_4 Hydrolysis & Colorimetry
Persulfate Digestion & Colorimetry
Soluble Orthophosphate
Soluble Hydrolyzable & Orthophosphate
Total Soluble Phosphorus
inorganic phosphorus compounds
polyphosphate + some organic phosphorus compounds
inorganic + organic phosphorus compounds

FIGURE 1

ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS

B Interferences

1 Erroneous results from contaminated glassware is avoided by cleaning it with hot 1:1 HCl, treating it with procedure reagents and rinsings with distilled water. Preferably this glassware should be used only for the determination of phosphorus and protected from dust during storage. Commercial detergents should never be used.

2 High iron concentrations in samples can precipitate phosphorus.

3 If $HgCl_2$ is used as a preservative, it interferes if the chloride level of the sample is less than 50 mg Cl/liter. Spiking with NaCl is then recommended.

4 Others have reported interference from chlorine, chromium, sulfides, nitrite, tannins, lignin and other minerals and organics at high concentrations.

C Precision and Accuracy(6)

1 Organic phosphate - 33 analysts in 19 laboratories analyzed natural water samples containing exact increments of organic phosphate of 0.110, 0.132, 0.772, and 0.882 mg P/liter.

Standard deviations obtained were 0.033, 0.051, 0.130 and 0.128 respectively.

Accuracy results as bias, mg P/liter were: +0.003, +0.016, +0.023 and - 0.008, respectively.

2 Orthophosphate was determined by 26 analysts in 16 laboratories using samples containing orthophosphate in amounts of 0.029, 0.038, 0.335 and 0.383 mg P/liter.

Standard deviations obtained were 0.010, 0.008, 0.018 and 0.023 respectively.

Accuracy results as bias, mg P/liter were -0.001, -0.002, -0.009 and -0.007 respectively.

D Automated Methods

The EPA Manual also contains a procedure for an automated colorimetry method using the ascorbic acid reduction method.

VII VARIABLES IN THE COLORIMETRIC PROCEDURE

Several important variables affect formation of the yellow heteropoly acid and its reduced form, molybdenum blue, in the colorimetric test for P.

A Acid Concentration during color development is critical. Figure 2 shows that color will appear in a sample containing no phosphate if the acid concentration is low. Interfering color is negligible when the normality with respect to H_2SO_4 approaches 0.4.

1 Acid normality during color development of 0.3 to slightly more than 0.4 is feasible for use. It is preferable to control acidity carefully and to seek a normality closer to the higher limits of the acceptable range.

2 It is essential to add the acid and molybdate as one solution.

3 The aliquot of sample must be neutralized prior to adding the color reagent.

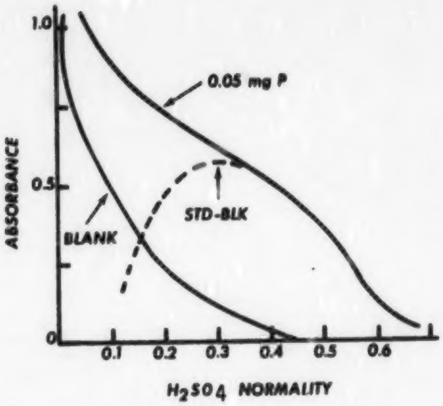


Figure 2

0-PHOSPHATE COLOR VS ACIDITY

B Choice of Reductant - Reagent stability, effective reduction and freedom from deleterious side effects are the bases for reductant selection. Several reductants have been used effectively. Ascorbic acid reduction is highly effective in both marine and fresh water. It is the reductant specified in the EPA method.

C Temperature affects the rate of color formation. Blank, standards, and samples must be adjusted to the same temperature (± 1°C), (preferably room temperature), before addition of the acid molybdate reagent.

D Time for Color Development must be specified and consistent. After addition of reductant, the blue color develops rapidly for 10 minutes then fades gradually after 12 minutes.

VIII DETERMINATION OF TOTAL PHOSPHORUS

A Determination of total phosphorus content involves omission of any filtration procedure and using the acid-hydrolysis and persulfate treatments to convert all phosphorus forms to the test-sensitive orthophosphate form.

B Determining total phosphorus content yields the most meaningful data since the various forms of phosphorus may change from one form to another in a short period of time. (See part V, B1)

IX DEVELOPMENT OF A STANDARD PROCEDURE

Phosphorus analysis received intensive investigation; coordination and validation of methods is more difficult than changing technique.

A Part of the problem in methods arose because of changes in analytical objectives such as:

1 Methods suitable to gather "survey" information may not be adequate for "standards".

2 Methods acceptable for water are not necessarily effective in the presence of significant mineral and organic interference characteristic of hydrosoils, marine samples, organic sludges and benthic deposits.

3 Interest has been centered on "fresh" water. It was essential to extend them for marine waters.

4 Instrumentation and automation have required adaptation of methodology.

B Analysts have tended to work on their own special problems. If the method apparently served their situations, it was used. Each has a "favorite" scheme that may be quite effective but progress toward widespread application of "one" method has been slow. Consequently, many methods are available. Reagent acidity, Mo content, reductant and separation techniques are the major variables.

C At the present time there is not sufficient data to warrant EPA endorsement of the P procedure for sediment-type samples, sludges, algal blooms, etc. Following is a procedure (not included in the EPA manual) which is useful when solids are present in samples:

1 If sample contains large particles, grind and emulsify solids in a blender.

2 Transfer 50 ml sample, or aliquot diluted to 50 ml, into a 250 ml Erlenmeyer flask.

3 Add 6.0 ml of 18N H_2SO_4, 5 ml concentrated HNO_3, 2 berl saddles and digest on hot plate.

4 Digest until the disappearance of nitric acid fumes and the appearance of white SO_3 fumes. Continue digestion for approximately 5 minutes. Cool before proceeding with Step 5.

5 Add 2 ml of HNO_3-$HClO_4$ mixture and 5 ml concentrated HNO_3. Continue digestion until all of the nitric acid is driven off and dense fumes of perchloric evolve. Perchloric acid requires dilution with sulfuric acid and prior destruction of most organics for safety.

6 Cool. Add approximately 40 ml distilled water and transfer to 100 ml volumetric flask.

7 Add 2-3 drops of phenolphthalein and concentrated ammonium hydroxide until a pink color is seen. Then discharge the pink color with the strong sulfuric acid. It is advisable to add an equivalent amount of salt formed during neutralization of digested samples to the calibration standards to equalize salt content during color development.

8 Determine orthophosphate according to the usual color procedure.

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REFERENCES

1 Jenkins, David, A Study of Methods Suitable for the Analysis and Preservation of Phosphorus Forms in the Estuarine Environment. DHEW, Central Pacific River Basins Project. SERL Report No. 65-18, University of California, Berkeley, Calif. November 1965.

2 Gales, Morris E., Jr., Julian, Elmo C., and Kroner, Robert C., Method for Quantitative Determination of Total Phosphorus in Water. JAWWA 58: (10) 1363. October 1966.

3 Lee, G. Fred, Clesceri, Nicholas L. and Fitzgerald, George P., Studies on the Analysis of Phosphates in Algal Cultures. Int. J. Air & Water Poll. 9:715. 1965.

4 Barth, E. F. and Salotto, V. V., Procedure for Total Phosphorus in Sewage and Sludge, Unpublished Memo, Cincinnati Water Research Laboratory, FWQA. April 1966.

5 Moss, H. V., (Chairman, AASGP Committee) Determination of Ortho Phosphate, Hydrolyzable Phosphate and Total Phosphate in Surface Water. JAWWA 56:1563. December 1958.

6 Methods for Chemical Analysis of Water & Wastes, EPA, MDQARL, Cincinnati,Ohio 45268, 1974.

This outline was prepared by Ferdinand J. Ludzack, Chemist, Audrey E. Donahue, Chemist, both with National Training Center, MPOD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors:
Chemical Analysis, Nutrients, Phosphates, Phosphorus, Phosphorus Compounds, Pollutant Identification, Sampling, Water Analysis, Water Pollution Sources.

BIOCHEMICAL OXYGEN DEMAND TEST
DILUTION TECHNIQUE

I **OBJECTIVES:** to prepare a sample dilution for series observation of oxygen depletion versus time for the estimation of BOD$_t$, rate and ultimate demand. The azide modification of the Winkler iodometric titration of dissolved oxygen will be used. An electronic DO analyzer will be used to check the titrimetric DO.

II **PROCEDURE:**

A Each group will prepare 1 dilution of either effluent or influent at an assigned concentration to enable filling 7 standard BOD bottles. One of these will be used for electronic DO monitoring by the lab instructor, the other six are for daily student determinations. Identify each bottle with group number, I for influent or E for effluent and the assigned concentration in %.

B Calculate the volume of sample to be used to make up a total diluted volume of 3500 ml at the assigned concentration. Then dilute the sample as follows:

1 Add dilution water to the dilution bottle to make up about 1/2 of the total amount of distilled water to be used. Add 1 ml each of the four mineral supplements. (These solutions are prepared at 4 times the concentration of Standard Methods mineral supplement reagents so the added volume is lower). Mix the supplemented dilution water thoroughly.

2 Carefully mix the bottle of sample to be used to a uniform suspension. If large chunks are evident, homogenize. Rapidly pour out, with mixing, into a graduate, the amount of sample required and return to lab station.

(Mixing, time, and precision are important. Your sample may be non-representative to the extent of carelessness in mixing, allowing settling while adjusting measurement or measurement errors.)

3 Add the measured quantity of sample to the dilution bottle.

4 Carefully add dilution water to make up 3500 ml of diluted sample. Use some of the dilution water to rinse the graduate into the bottle.

5 Stopper and shake the dilution bottle to mix the sample and to ensure that you have an acceptable initial DO. This step will add DO or reduce supersaturation. About 15 seconds of mixing is usually adequate.

C Filling bottles is simplified if you place the dilution bottle on the center pedestal, insert the siphon and line up your identified bottles with stoppers removed. Have a container larger than the bottles available to accommodate the overflow while filling them. Otherwise, move the apparatus near a sink. Fill the siphon tube and waste some of the diluted sample to rinse this tube. Be sure that the siphon tube is used in a manner so that it is always full of the diluted sample. Agitate the diluted sample regularly to avoid undue collection of solids on the bottom.

1 Place first bottle to be filled in the overflow container or hold over sink. Insert the siphon delivery tip all of the way down into the bottom of the test bottle. Tilt the bottle to cover the delivery tip with water promptly. Start the siphon flow slowly to decrease turbulence until the delivery tip is well-covered. Fill to overflowing. Slowly raise the delivery tip while overflowing the sample. Stop the flow with the bottle completely full. Insert the bottle stopper and turn it slightly to seat it. Fill the remaining bottles in the same manner.

2 Each bottle will have the same DO and sample mix as the next if you have followed a consistent procedure, mixed carefully, and have not changed technique enroute.

3 One of the seven bottles is to be retained for initial titration of DO by your group. Another bottle is to be turned in to the lab instructor for use in performance checks on all class dilutions. The remainder are to be placed into trays for 20° incubation. Be certain that all bottles are properly identified.

D Dissolved Oxygen Determination

1 You are encouraged to check the DO of your sample on the electronic analyzer. A calibrated unit will be available with assistance. The DO analyzer will not result in more than 1/2 ml loss of sample on probe insertion. You will be able to add DO reagents and continue the Winkler titrimetric DO on the same bottle.

2 An initial DO value (DO_o) using the Winkler method must be determined today. On succeeding days, the DO_t will be determined on the incubated samples for estimation of BOD progression. (If reaeration is required, the technique will be demonstrated). All data is to be recorded on the sheet provided in this manual.

The Winkler-Azide procedure as presented in outline 17 is to be used for these DO determinations.

3 A blank value for each stock bottle of distilled water will be determined by the staff.

REFERENCE

Standard Methods for the Examination of Water and Wastewater, 13th edition. APHA, AWWA, WPCF, New York. p. 489. 1971.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Analytical Techniques, Biochemical Oxygen Demand, Chemical Analysis, Laboratory Tests, Water Analysis

BOD DATA SHEET

Group No.__________ **Initial Date**______________

Buret Readings	DO_o	Buret Readings	DO_t	ΔDO	Sf/ID	BOD

Buret readings - initial and final

DO_o - initial DO in mg/liter

DO_t = DO at indicated incubation time $_t$

ΔDO = DO change (DO_o - DO_t); a depletion

Sf = the sample content as a decimal fraction in the dilution

ID = sample identification (influent or effluent)

BOD = $\frac{DO_o - DO_t}{Sf}$ (according to Standard Methods, 13th edition, 1971)

LABORATORY PROCEDURE FOR DISSOLVED OXYGEN

Winkler-Azide Procedure

I INTRODUCTION

The azide modification is used for most sewage, effluents, and streams which contain nitrate nitrogen and not more than 1 mg of ferrous iron/l. If 1 ml 40% KF solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100-200 mg/l ferric iron.

The "Methods for Chemical Analysis of Water & Wastes, 1974," published by the Environmental Protection Agency, recommends the Winkler-Azide method using the full bottle technique.

II REAGENTS

A Manganous Sulfate Solution

Dissolve 480 g $MnSO_4 \cdot 4H_2O$ in distilled water and dilute to 1 liter.

B Alkali-Iodide-Azide Reagent

Dissolve 500 g sodium hydroxide and 150 g potassium iodide in distilled water and dilute to 1 liter. To this solution add 10 g sodium azide. NaN_3 dissolved in 40 ml distilled water.

C Sulfuric Acid, Conc.

The strength of this acid is 36 N.

D Starch Solution

Prepare an emulsion of 10 g of soluble starch in a mortar or beaker with a small quantity of distilled water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 5 ml per liter of chloroform and storage in a 10° C refrigerator.

E Sodium Thiosulfate Stock Solution 0.75 N

Dissolve 186.15 g $Na_2S_2O_3 \cdot 5H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 ml chloroform.

F Sodium Thiosulfate Standard Titrant 0.0375N

Dilute 50.0 ml of stock solution to 1 liter. Preserve by adding 5 ml of chloroform. Standardize with potassium biiodate.

III PROCEDURE

A Addition of Reagents

1 Manganous sulfate and alkali-iodide-azide

To a full sample bottle (300 ml ± 3 ml BOD incubation), add 2 ml manganous sulfate solution and 2 ml alkaline-iodide reagent with the tip of each pipette below the surface of the sample.

2 Replace the stopper.

3 Rinse under running water.

4 Mix well by inverting 4-5 times.

5 Allow the precipitate to settle until at least 100 ml of clear supernate have been produced.

6 Repeat steps 4 and 5.

7 Add 2 ml conc. sulfuric acid with the tip of the pipette above the surface of the sample.

8 Stopper the bottle.

9 Rinse under running water.

10 Mix by inverting several times to dissolve the precipitate.

11 Pour contents of bottle into a wide mouth 500 ml Erlenmeyer flask.

B Titration

1 Titrate with 0.0375 N thiosulfate to a pale straw color.

2 Add 2 ml starch solution indicator.

3 Continue titrating to the disappearance of the blue color.

C Calculation

$$\frac{\text{mls titrant} \times 0.0375 \times 8000}{300} = \text{mg/l DO}$$

$$\text{mls titrant} \times 0.0125 \times 80 = \text{mg/l DO}$$

$$\text{mls titrant} \times 1 = \text{mg/l DO}$$

$$\text{mls titrant} = \text{mg/l DO}$$

REFERENCE

Methods for Chemical Analysis of Water and Wastes. EPA-MDQARL, Cincinnati, Ohio, 1974.

This outline was prepared by J. W. Mandia, Chemist, formerly with National Training Center, and revised by C. R. Feldmann, Chemist, National Training Center, MPOD, OWPO, EPA, Cincinnati, OH 45268.

Descriptors: Analytical Techniques, Chemical Analysis, Dissolved Oxygen, Laboratory Tests, Oxygen, Water Analysis

DETERMINATION OF KJELDAHL NITROGEN
(MICRO APPARATUS-NESSLERIZATION)

I REAGENTS

A Distilled Water

This should be ammonia-free. Pass distilled water through an ion exchange column with strongly acidic cation resin mixed with a strongly basic anion resin.

B Sulfuric Acid (20%)

20 ml acid/100 ml distilled water

C Mercuric Sulfate Solution

Dissolve 8g mercuric oxide in 50 ml of 20% sulfuric acid. Dilute to 100 ml with distilled water.

D Digestion Reagent

Dissolve 134g potassium sulfate in about 650 ml distilled water. Add 200 ml concentrated sulfuric acid. Add 25 ml of mercuric sulfate solution (C above) and dilute to 1 liter.

E Sodium Hydroxide - Sodium Thiosulfate Solution

Dissolve 500g sodium hydroxide and 25g sodium thiosulfate pentahydrate in distilled water and dilute to 1 liter.

F Mixed Indicator

Mix 100 ml of methyl red indicator (200 mg in 100 ml 95% ethyl alcohol) with 50 ml of methylene blue indicator solution (200 mg in 100 ml 95% ethyl alcohol).

G Boric Acid Solution. 2%

Dissolve 20g boric acid in distilled water and dilute to 1 liter.

H Ammonium Chloride Stock Solution

Dissolve 3.819g NH_4Cl in distilled water and dilute to 1 liter. 1.0 ml = 1.0 mg NH_3-N.

I Ammonium Chloride Standard Solution

Dilute 10.0 ml of the stock solution with distilled water to 1 liter.
1.0 ml = 0.01 mg NH_3-N

J Sodium Hydroxide Solution

Dissolve 160 g sodium hydroxide in 500 ml distilled water.

K Nessler Reagent

Dissolve 100g mercuric iodide and 70g potassium iodide in a small volume of water. Add this mixture slowly to the sodium hydroxide solution (J above), then dilute to 1 liter.

II EQUIPMENT PREPARATION

A This procedure should be used if the apparatus has been out of service for 4 hours or more.

1 Add about 50 ml of a 1:1 mixture of ammonia-free distilled water and sodium hydroxide-sodium thiosulfate solution to each of the micro Kjeldahl flasks to be used.

2 Add glass beads to each flask.

3 Attach a flask to the steam distillation apparatus and distill about half the mixture.

4 Add 1 ml of the Nessler reagent to the distillate to check for ammonia.

a If the distillate is colorless, the equipment is ammonia-free and the procedure can be repeated with the next flask to be used.

b If the distillate is yellow, discard it, distill another half of the mixture and check this distillate with 1 ml Nessler reagent. Repeat the process until the distillate is colorless.

III DIGESTION OF SAMPLE

A Preparation of Digestion Mixture

1 Shake the sample.

2 Measure 50.0 ml sample into a 100 ml Kjeldahl flask.

3 Add 10 glass beads.

4 Add 10 ml of the digestion reagent.

B Digestion

1 Place the flask in a properly ventilated Kjeldahl digestion apparatus.

2 Turn on the heat source.

3 Evaporate the mixture until sulfur trioxide (SO_3) fumes are given off. (SO_3 fumes are white. Also, the solution will be pale yellow.)

4 Continue heating for an additional 30 minutes.

5 Turn off the heat source.

6 Cool the residue in the flask.

IV STEAM DISTILLATION

A Preparation of the Digestion Residue

1 Add 30 ml of ammonia-free distilled water to the digested residue in the Kjeldahl flask.

2 Connect the flask to the ground glass joint of the micro steam distillation apparatus.

3 Add 5 ml of 2% boric acid to a 50 ml Erlenmeyer receiving flask.

4 Position the Erlenmeyer flask so that the tip of the condenser is below the level of the boric acid solution in the receiving flask.

5 Carefully add 10 ml of the sodium hydroxide-sodium thiosulfate solution from the dropping funnel.

B Distillation

1 Turn on the heat source.

2 Distill at a rate of 6-10 ml/minute up to the 30 ml mark on the Erlenmeyer receiving flask.

3 Remove the receiving flask.

4 Put a small beaker under the condenser tip to receive any additional distillate.

5 Turn off the heat source if there are no more digestion residues to distill.

V COLORIMETRY FOR AMMONIA-NESSLERIZATION

If the ammonia content is found to be greater than 1 mg/liter, a titration procedure should be used(1) rather than Nesslerization.

A Preparation of Standards and Sample

1 Label nine 50.0 ml Nessler tubes with the following: 0, 0.5, 1, 2, 4, 5, 8, 10, and S.

2 Pipet the following volumes of ammonium chloride standard solution into the correspondingly labeled tubes: 0.5 ml, 1.0 ml, 2.0 ml, 4.0 ml, 5.0 ml, 8.0 ml, and 10.0 ml.

3 Pour the 30 ml of distillate collected from the sample into the Nessler tube marked "S".

4 Pour ammonia-free distilled water into the tube labeled "0", bringing the volume to the 50.0 ml line.

5 Add ammonia-free distilled water to each of the remaining 8 tubes, bringing the volume of each to the 50.0 ml line.

6 Add 1.0 ml of Nessler Reagent to each of the 9 tubes.

7 Mix the solution in the "0" tube using a cap or a rubber stopper on the top, then inverting the tube three times.

8 Remove, rinse, and dry the cap or stopper.

9 Repeat steps 7 and 8 to mix the contents of the other 8 tubes.

10 After mixing, let the tubes stand in a rack for 20 minutes before getting absorbance readings.

B Spectrophotometric Readings

1 Turn the instrument on.

2 Set the wavelength at 425 nm.

3 After the 20 minute time span, use the contents of the "0" tube to adjust to zero absorbance on the spectrophotometer.

4 Using the contents of the tube labeled 0.5, rinse, then fill an instrument cell.

5 Place the cell in the holder and record the absorbance value from the instrument.

6 Discard the contents from the cell.

7 Use ammonia-free distilled water to rinse the cell three times.

8 Repeat steps 4 through 7 to obtain absorbance values for the rest of the standards and for the sample.

9 Turn off the instrument.

VI CALIBRATION CURVE

A Constructing the Curve

1 Calculate the concentration of each standard by multiplying the ml of working standard used times 0.01 mg/ml, which is the concentration of the standard solution. This was diluted to 50.0 ml in the Nessler tube, so the result is mg NH_3-N/50.0 ml. For example, if 0.5 ml of standard was used, the concentration is (0.5)(0.01) = 0.005 mg NH_3-N/50.0 ml.

2 Plot the absorbance values for the standards against these calculated concentrations.

3 Draw the best straight line from zero through all the points.

B Using the Curve

1 To find the NH_3-N concentration in the sample, locate its absorbance value on the curve.

2 Find the corresponding mg NH_3-N/50.0 ml by dropping a vertical line to the concentration axis.

3 Record this result.

VII FINAL CALCULATIONS

A Use this formula to calculate Total Kjeldahl Nitrogen:

$$\text{TKN, mg/l} = \frac{A \times 1000}{\text{ml sample}} \times \frac{B}{C}$$

Where:

A = mg NH_3-N/50.0 ml from curve
B = ml total distillate including boric acid
C = ml distillate taken for Nesslerization
ml sample = ml of original sample taken

B An example calculation using the value from the calibration curve would be:

$$\text{TKN, mg/l} = \frac{A \times 1000}{\text{ml sample}} \times \frac{B}{C}$$

If:

A = 0.045
B = 30 ml (25 ml distillate + 5 ml boric acid)
C = 30 ml
ml sample = 50 ml

Then:

$$\text{TKN, mg/l} = \frac{0.045 \times \overset{20}{\cancel{1000}}}{\underset{1}{\cancel{50}}} \times \frac{\overset{1}{\cancel{10}}}{\underset{1}{\cancel{10}}}$$

$$= 0.045 \times 20 \times 1$$

$$= 0.045 \times 20$$

$$= 0.90$$

TKN = 0.90 mg/l

REFERENCE

1 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL. 1974.

This outline was prepared by A. E. Donahue, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Ammonia, Analytical Techniques, Chemical Analysis, Laboratory Tests, Nitrogen Nitrogen Compounds, Nutrients, Water Analysis

LABORATORY PROCEDURE FOR TOTAL SOLIDS

I INTRODUCTION

A This procedure was excerpted from methods for Chemical Analysis of Water and Wastes, 1971, Environmental Protection Agency, Office of Water Programs, Analytical Quality Control Laboratory.

B The procedure is applicable to surface and saline waters, domestic and industrial wastes.

C The practical range of the determination is 10-30000 mg/l.

D Nonhomogenous materials (large floating particles or submerged agglomerates) should be excluded from the sample. Floating grease and oil should be included in the sample and dispersed in a blender before measuring the aliquot.

E Samples should be analyzed as soon as possible.

II EQUIPMENT

A Porcelain, Vycor, or platinum evaporating dishes, 100 ml capacity; smaller sizes may be used as required.

B Muffle furnace, 550 ± 50°C

C Drying oven, 103 - 105°C

D Desiccator

E Analytical balance

F Steam bath or Oven at 98°C

III PROCEDURE

A Heat the clean evaporating dish in a muffle furnace at 550 ± 50°C for 1 hour.

B Cool the dish in a desiccator.

C Store the dish in the desiccator and weigh just before use.

D Weigh the dish on an analytical balance.

E Shake the sample container vigorously.

F Measure 100 ml of the well mixed sample in a graduated cylinder. (At least 25 mg of residue should be obtained; less volume of sample may be used if the sample appears to be high in solids content. If it is low in solids content, more sample may be added to the dish after drying).

G Rapidly, but without spilling, pour the sample into the evaporating dish.

H Dry the sample on a steam bath, or at 98°C (to prevent boiling and splattering) in the oven.

I Dry the evaporated sample in the oven at 103 - 105°C for at least one hour.

J Cool the dish in the desiccator and then weigh it.

K Repeat the heating at 103 - 105°C, cooling and weighing until the weight loss is less than 4% of the previous weight, or 0.5 mg, whichever is less.

IV CALCULATIONS

$$\text{mg total solids/l} = \frac{(\text{wt dish + residue})^* - (\text{wt dish})^* \times 1000 \times 1000}{\text{ml of sample}}$$

* in grams

This outline was prepared by C. R. Feldmann, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Analytical Techniques, Chemical Analysis, Laboratory Tests, Solids, Water Analysis

LABORATORY PROCEDURE FOR TOTAL HARDNESS

I REAGENTS

A Buffer Solution:

1 Dissolve 16.9 g of NH_4Cl in 143 ml of conc. NH_4OH.

2 Dissolve 1.179 g of analytical reagent grade disodium ethylenediamine tetraacetic acid dihydrate($Na_2EDTA \cdot 2H_2O$) and 0.644 g of $MgCl_2 \cdot 6H_2O$ in 50 ml of distilled water.

3 Add the solution from (2) to the solution from (1) with mixing, and dilute to 250 ml with distilled water. Addition of small amounts of $Na_2EDTA \cdot 2H_2O$ or $MgCl_2 \cdot 6H_2O$ may be necessary to attain exact equivalence.

4 The buffer should be stored in a plastic or resistant glass container tightly stoppered to prevent CO_2 absorption and NH_3 loss. Discard the buffer when 1 or 2 ml added to the sample fails to produce a pH of 10.0 ± 0.1 at the end point of the titration.

B Inhibitor:

1 Most water samples do not require the use of an inhibitor. In the presence of certain interferring ions, however, an inhibitor may be needed to sharpen the endpoint color change. Several types of inhibitors may be prepared or purchased. Sodium cyanide, NaCN, is one of the simpler inhibitors to use.

2 Add 0.25 g of powdered NaCN to the sample and adjust the pH to 9.9-10.1. Caution: NaCN is poisonous. Use large amounts of water when flushing solutions containing NaCN down the drain. Do not acidify solutions containing NaCN; volatile, poisonous hydrogen cyanide, HCN, would be liberated.

C Indicator:

1 Eriochrome Black-T dye (EBT) is useful for the determination. Other commercial grades or laboratory formulations of the dye are also satisfactory.

2 Prepare the indicator in dry powder form by grinding together 0.5 g of the dye and 100 g of NaCl.

D Standard Calcium Carbonate, $CaCO_3$:

Weigh 1.000 g of anhydrous, primary standard grade $CaCO_3$ and transfer it to a 500 ml Erlenmeyer flask. Add 1:1 HCl (equal volumes of conc. HCl and water) dropwise and with swirling of the flask until the $CaCO_3$ has dissolved. Bring the volume of liquid to about 200 ml with water, boil a few seconds to dispel CO_2, cool, and add a few drops of methyl red indicator. Adjust the color of the solution to an intermediate orange by the dropwise addition of 1:1 HCl or 1:4 NH_4OH (1 volume of conc. NH_4OH + 4 volumes of water). Transfer the solution quantitatively to a one liter volumetric flask and dilute to the mark with water. (1.0 ml = 1.0 mg$CaCO_3$)

E $Na_2EDTA \cdot 2H_2O$ (0.01 M):

Dissolve 3.723 g of the dry reagent grade $Na_2EDTA \cdot 2H_2O$ in distilled water and dilute to 1 liter. 1.0 ml of the 0.01 M solution = 1.0 mg of $CaCO_3$. Check the concentration of this solution by titration against the standard calcium carbonate solution as described in II below.

II STANDARDIZATION OF THE $Na_2EDTA \cdot 2H_2O$:

A Dilute 25.0 ml of $CaCO_3$ standard to about 50 ml with distilled water in a 125 ml Erlenmeyer flask against a white background.

B Add 1-2 ml of the buffer solution and check the pH to ensure that it is 9.9 to 10.1.

C Add approximately 0.2 g of the indicator.

D Add the $Na_2EDTA \cdot 2H_2O$ slowly and with stirring until the color changes from a rose to a blue color. If the color change is not sharp, repeat the determination using the inhibitor. If the endpoint is still not sharp, prepare a fresh supply of indicator.

E The titration should take less than 5 minutes, measured from the time of buffer addition.

F In an analysis of this type it is advantageous to carry out a preliminary, rapid titration in order to determine approximately how much titrant will be required. This is accomplished by adding the $Na_2EDTA \cdot 2H_2O$ at a fast dropwise rate until the color change is observed.

III PROCEDURE

Repeat steps II A through II F using sample in place of $CaCO_3$ standard. The amount of sample taken should require less than 15 ml of $Na_2EDTA \cdot 2H_2O$ titrant.

IV CALCULATION

A Standardization of the $Na_2EDTA \cdot 2H_2O$:

ml of $CaCO_3$ equal to 1.0 ml of the $Na_2EDTA \cdot 2H_2O$

$$\text{(symbol B)} = \frac{\text{ml of } CaCO_3}{\text{ml of } Na_2EDTA \cdot 2H_2O \text{ required for titration}}$$

B Total Hardness

$$\text{Hardness as mg } CaCO_3/l = \frac{A \times B \times 1000}{\text{ml of sample}}$$

A = ml of $Na_2EDTA \cdot 2H_2O$ for titration of sample

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater. 13th Edition, A PHA, -AWWA-WPCF (1971) p. 179, Method 122B.

2 ASTM Standards, Part 23, Water; Atmospheric Analysis 1972 p. 170, Method D1126-67.

3 Methods for Chemical Analysis of Water and Wastes, 1974, Environmental Protection Agency, MDQARL, Cincinnati, OH.

This outline was prepared by C. R. Feldmann, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Analytical Techniques, Calcium, Chemical Analysis, Hardness, Laboratory Tests, Magnesium, Water Analysis

ACIDITY

1. Scope and Application

1.1 This method is applicable to surface waters, sewages and industrial wastes, particularly mine drainage and receiving streams, and other waters containing ferrous iron or other polyvalent cations in a reduced state.

1.2 The method covers the range from approximately 10 mg/l acidity to approximately 1000 mg/l as $CaCO_3$, using a 50 ml sample.

2. Summary of Method

2.1 The pH of the sample is determined and a measured amount of standard acid is added, as needed, to lower the pH to 4 or less. Hydrogen peroxide is added, the solution boiled for several minutes, cooled, and titrated electrometrically with standard alkali to pH 8.2.

3. Definitions

3.1 This method measures the mineral acidity of a sample plus the acidity resulting from oxidation and hydrolysis of polyvalent cations, including salts of iron and aluminum.

4. Interferences

4.1 Suspended matter present in the sample, or precipitates formed during the titration may cause a sluggish electrode response. This may be offset by allowing a 15-20 second pause between additions of titrant or by slow dropwise addition of titrant as the endpoint pH is approached.

5. Apparatus

5.1 pH meter, suitable for electrometric titrations.

6. Reagents

6.1 Hydrogen peroxide (H_2O_2, 30% solution).

6.2 Standard sodium hydroxide, 0.02 N.

6.3 Standard sulfuric acid, 0.02 N.

7. Procedure

7.1 Pipet 50 ml of the sample into a 250 ml beaker.

7.2 Measure the pH of the sample. If the pH is above 4.0 add standard sulfuric acid in 5.0 ml increments to lower the pH to 4.0 or less. If the initial pH of the sample is less than 4.0, the incremental addition of sulfuric acid is not required.

7.3 Add 5 drops of hydrogen peroxide.

7.4 Heat the sample to boiling and continue boiling for 2 to 4 minutes. In some instances, the concentration of ferrous iron in a sample is such that an additional amount of hydrogen peroxide and a slightly longer boiling time may be required.

7.5 Cool the sample to room temperature and titrate electrometrically with standard alkali to pH 8.2.

8. Calculations

8.1 Acidity, as mg/l $CaCO_3 = \frac{(A \times B) - (C \times D) \times 50,000}{\text{ml sample}}$

where:

A = vol. of standard alkali used in titration

B = normality of standard alkali

C = volume of standard acid used to reduce pH to 4 or less

D = normality of standard acid

8.2 If it is desired to report acidity in millequivalents per liter, the reported values as $CaCO_3$ are divided by 50, as follows:

$$\text{Acidity as meq/l} = \frac{\text{mg/l } CaCO_3}{50}$$

9. Precision

9.1 On a round robin conducted by ASTM on 4 acid mine waters, including concentrations up to 2000 mg/l, the precision was found to be ±10 mg/l.

10. References

10.1 The procedure to be used for this determination can be found in:

ASTM Standards, Part 23, Water; Atmospheric Analysis, p 124, D-1067, Method E (1973).

Standard Methods for the Examination of Water and Wastewater, 13th Edition, p 370, Method 201 (Acidity and Alkalinity) (1971).

(The preceding is a copy of pp 1 and 2 from Methods for Chemical Analysis of Water and Wastes. EPA-MDQARL. 1974.)

Descriptors: Acids, Acidity, Analytical Techniques, Chemical Analysis, Laboratory Tests, Neutralization, Water Analysis

LABORATORY PROCEDURE FOR TOTAL ALKALINITY

I REAGENTS

For detailed discussion of reagent preparation, consult method reference 3.

A Carbon Dioxide - Free Distilled Water

B Standard Sodium Carbonate Solution

C Hydrochloric Acid Titrant (0.02 N)

II STANDARDIZATION OF THE HYDROCHLORIC ACID TITRANT

A Set the temperature reading on the pH meter dial to match the temperature of the buffer and sample solutions.

B Standardize the pH meter against a reference buffer solution. Check against a second buffer solution.

C Weigh accurately 0.088 ± 0.001 g of the dried sodium carbonate and transfer it to a 500 ml conical flask.

D Add 50 ml of water and swirl to dissolve the carbonate.

E While stirring the solution (magnetic bar and stirrer), add the hydrochloric acid titrant from a 100 ml buret until a pH of 4.5 is attained.

F Calculate the normality of the hydrochloric acid solution as follows:

$$A = \frac{B}{0.053 \times C}$$

A = normality of the hydrochloric acid

B = g of sodium carbonate used

C = ml of hydrochloric acid consumed

0.053 = milliequivalent weight of Na_2CO_3

III PROCEDURE

A Pipette 50 ml of the sample into a 150 ml beaker.

B Titrate with the hydrochloric acid to pH 4.5.

C Calculation

Total alkalinity as mg of $CaCO_3/l$ =

$$\frac{A \times N \times 50000}{B}$$

A = ml of standard HCl titrant

N = N of standard HCl titrant

B = ml of sample

50 = equivalent weight of $CaCO_3$

1000 - converts ml to liters

REFERENCES

1 Methods for Chemical Analysis of Water and Wastes, EPA-MDQARL, Cincinnati, Ohio 45268, 1974, p. 3.

2 Standard Methods for the Examination of Water and Wastewater, 13th ed., 1971, p. 370.

3 Book of ASTM Standards Part 23, 1972, p. 143.

This outline was prepared by C. R. Feldmann, Chemist, National Training Center, MPOD, OWPO, USEPA, Cincinnati, Ohio 45268.

Descriptors: Alkalis, Alkalinity, Analytical Techniques, Chemical Analysis, Laboratory Tests, Neutralization, Water Analysis